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(54) Title: PLATELET–DERIVED GROWTH FACTOR C, DNA CODING THEREFOR, AND USES THEREOF

## PLATELET-DERIVED GROWTH FACTOR C, DNA CODING THEREFOR, AND USES THEREOF

This invention relates to growth factors for connective tissue cells, fibroblasts, myofibroblasts and glial cells and/or to growth factors for endothelial cells, and in particular to a novel platelet-derived growth factor/vascular

5     endothelial growth factor-like growth factor, a polynucleotide sequence encoding the factor, and to pharmaceutical and diagnostic compositions and methods utilizing or derived from the factor.

10     BACKGROUND OF THE INVENTION

In the developing embryo, the primary vascular network is established by *in situ* differentiation of mesodermal cells in a process called vasculogenesis. It is believed that all subsequent processes involving the generation of new vessels

15     in the embryo and neovascularization in adults, are governed by the sprouting or splitting of new capillaries from the pre-existing vasculature in a process called angiogenesis (Pepper *et al.*, Enzyme & Protein, 1996 <u>49</u> 138-162; Breier *et al.*, Dev. Dyn. 1995 <u>204</u> 228-239; Risau, Nature, 1997 <u>386</u>

20     671-674). Angiogenesis is not only involved in embryonic development and normal tissue growth, repair, and regeneration, but is also involved in the female reproductive cycle, establishment and maintenance of pregnancy, and in repair of wounds and fractures. In addition to angiogenesis

25     which takes place in the normal individual, angiogenic events are involved in a number of pathological processes, notably tumor growth and metastasis, and other conditions in which blood vessel proliferation, especially of the microvascular system, is increased, such as diabetic retinopathy, psoriasis

30     and arthropathies. Inhibition of angiogenesis is useful in preventing or alleviating these pathological processes.

On the other hand, promotion of angiogenesis is desirable in situations where vascularization is to be established or

extended, for example after tissue or organ transplantation, or to stimulate establishment of collateral circulation in tissue infarction or arterial stenosis, such as in coronary heart disease and thromboangitis obliterans.

5        The angiogenic process is highly complex and involves the maintenance of the endothelial cells in the cell cycle, degradation of the extracellular matrix, migration and invasion of the surrounding tissue and finally, tube formation.  The molecular mechanisms underlying the complex

10       angiogenic processes are far from being understood.

Because of the crucial role of angiogenesis in so many physiological and pathological processes, factors involved in the control of angiogenesis have been intensively investigated.  A number of growth factors have been shown to

15       be involved in the regulation of angiogenesis; these include fibroblast growth factors (FGFs), platelet-derived growth factor (PDGF), transforming growth factor alpha (TGFα), and hepatocyte growth factor (HGF).  See for example Folkman et al., J. Biol. Chem., 1992 267 10931-10934 for a review.

20       It has been suggested that a particular family of endothelial cell-specific growth factors, the vascular endothelial growth factors (VEGFs), and their corresponding receptors is primarily responsible for stimulation of endothelial cell growth and differentiation, and for certain

25       functions of the differentiated cells.  These factors are members of the PDGF family, and appear to act primarily via endothelial receptor tyrosine kinases (RTKs).

Nine different proteins have been identified in the PDGF family, namely two PDGFs (A and B), VEGF and six members that

30       are closely related to VEGF.  The six members closely related to VEGF are: VEGF-B, described in International Patent Application PCT/US96/02957 (WO 96/26736) and in U.S. Patents 5,840,693 and 5,607,918 by Ludwig Institute for Cancer

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Research and The University of Helsinki; VEGF-C, described in
Joukov et al., EMBO J., 1996 15 290-298 and Lee et al., Proc.
Natl. Acad. Sci. USA, 1996 93 1988-1992; VEGF-D, described in
International Patent Application No. PCT/US97/14696 (WO
98/07832), and Achen et al., Proc. Natl. Acad. Sci. USA, 1998
95 548-553; the placenta growth factor (PlGF), described in
Maglione et al., Proc. Natl. Acad. Sci. USA, 1991 88 9267-
9271; VEGF2, described in International Patent Application No.
PCT/US94/05291 (WO 95/24473) by Human Genome Sciences, Inc;
and VEGF3, described in International Patent Application No.
PCT/US95/07283 (WO 96/39421) by Human Genome Sciences, Inc.
Each VEGF family member has between 30% and 45% amino acid
sequence identity with VEGF.  The VEGF family members share
a VEGF homology domain which contains the six cysteine
residues which form the cysteine knot motif.  Functional
characteristics of the VEGF family include varying degrees of
mitogenicity for endothelial cells, induction of vascular
permeability and angiogenic and lymphangiogenic properties.

Vascular endothelial growth factor (VEGF) is a
homodimeric glycoprotein that has been isolated from several
sources.  VEGF shows highly specific mitogenic activity for
endothelial cells.  VEGF has important regulatory functions
in the formation of new blood vessels during embryonic
vasculogenesis and in angiogenesis during adult life
(Carmeliet et al., Nature, 1996 380 435-439; Ferrara et al.,
Nature, 1996 380 439-442; reviewed in Ferrara and Davis-Smyth,
Endocrine Rev., 1997 18 4-25).  The significance of the role
played by VEGF has been demonstrated in studies showing that
inactivation of a single VEGF allele results in embryonic
lethality due to failed development of the vasculature
(Carmeliet et al., Nature, 1996 380 435-439; Ferrara et al.,
Nature, 1996 380 439-442).  In addition VEGF has strong
chemoattractant activity towards monocytes, can induce the

plasminogen activator and the plasminogen activator inhibitor
in endothelial cells, and can also induce microvascular
permeability. Because of the latter activity, it is sometimes
referred to as vascular permeability factor (VPF). The
5    isolation and properties of VEGF have been reviewed; see
Ferrara et al., J. Cellular Biochem., 1991 47 211-218 and
Connolly, J. Cellular Biochem., 1991 47 219-223. Alterative
mRNA splicing of a single VEGF gene gives rise to five
isoforms of VEGF.

10       VEGF-B has similar angiogenic and other properties to
those of VEGF, but is distributed and expressed in tissues
differently from VEGF. In particular, VEGF-B is very strongly
expressed in heart, and only weakly in lung, whereas the
reverse is the case for VEGF. This suggests that VEGF and
15   VEGF-B, despite the fact that they are co-expressed in many
tissues, may have functional differences.

       VEGF-B was isolated using a yeast co-hybrid interaction
trap screening technique by screening for cellular proteins
which might interact with cellular resinoid acid-binding
20   protein type I (CRABP-I). Its isolation and characteristics
are described in detail in PCT/US96/02957 and in Olofsson et
al., Proc. Natl. Acad. Sci. USA, 1996 93 2576-2581.

       VEGF-C was isolated from conditioned media of the PC-3
prostate adenocarcinoma cell line (CRL1435) by screening for
25   ability of the medium to produce tyrosine phosphorylation of
the endothelial cell-specific receptor tyrosine kinase VEGFR-3
(Flt4), using cells transfected to express VEGFR-3. VEGF-C
was purified using affinity chromatography with recombinant
VEGFR-3, and was cloned from a PC-3 cDNA library. Its
30   isolation and characteristics are described in detail in
Joukov et al., EMBO J., 1996 15 290-298.

       VEGF-D was isolated from a human breast cDNA library,
commercially available from Clontech, by screening with an

expressed sequence tag obtained from a human cDNA library
designated "Soares Breast 3NbHBst" as a hybridization probe
(Achen *et al.*, Proc. Natl. Acad. Sci. USA, 1998 95 548-553).
Its isolation and characteristics are described in detail in
5      International     Patent     Application    No.    PCT/US97/14696
(WO98/07832).

The VEGF-D gene is broadly expressed in the adult human,
but is certainly not ubiquitously expressed.    VEGF-D is
strongly expressed in heart, lung and skeletal muscle.
10     Intermediate levels of VEGF-D are expressed in spleen, ovary,
small intestine and colon, and a lower expression occurs in
kidney, pancreas, thymus, prostate and testis.   No VEGF-D mRNA
was detected in RNA from brain, placenta, liver or peripheral
blood leukocytes.

15     PlGF was isolated from a term placenta cDNA library. Its
isolation and characteristics are described in detail in
Maglione *et al.*, Proc. Natl. Acad. Sci. USA, 1991 88 9267-
9271.    Presently its biological function is not well
understood.

20     VEGF2 was isolated from a highly tumorgenic, oestrogen-
independent human breast cancer cell line.    While this
molecule is stated to have about 22% homology to PDGF and 30%
homology to VEGF, the method of isolation of the gene encoding
VEGF2 is unclear, and no characterization of the biological
25     activity is disclosed.

VEGF3 was isolated from a cDNA library derived from colon
tissue.   VEGF3 is stated to have about 36% identity and 66%
similarity to VEGF.    The method of isolation of the gene
encoding VEGF3 is unclear and no characterization of the
30     biological activity is disclosed.

Similarity between two proteins is determined by
comparing the amino acid sequence and conserved amino acid
substitutions of one of the proteins to the sequence of the

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second protein, whereas identity is determined without including the conserved amino acid substitutions.

PDGF/VEGF family members act primarily by binding to receptor tyrosine kinases. Five endothelial cell-specific receptor tyrosine kinases have been identified, namely VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), VEGFR-3 (Flt4), Tie and Tek/Tie-2. All of these have the intrinsic tyrosine kinase activity which is necessary for signal transduction. The essential, specific role in vasculogenesis and angiogenesis of VEGFR-1, VEGFR-2, VEGFR-3, Tie and Tek/Tie-2 has been demonstrated by targeted mutations inactivating these receptors in mouse embryos.

The only receptor tyrosine kinases known to bind VEGFs are VEGFR-1, VEGFR-2 and VEGFR-3. VEGFR-1 and VEGFR-2 bind VEGF with high affinity, and VEGFR-1 also binds VEGF-B and PlGF. VEGF-C has been shown to be the ligand for VEGFR-3, and it also activates VEGFR-2 (Joukov *et al.*, The EMBO Journal, 1996 15 290-298). VEGF-D binds to both VEGFR-2 and VEGFR-3. A ligand for Tek/Tie-2 has been described in International Patent Application No. PCT/US95/12935 (WO 96/11269) by Regeneron Pharmaceuticals, Inc. The ligand for Tie has not yet been identified.

Recently, a novel 130-135 kDa VEGF isoform specific receptor has been purified and cloned (Soker *et al.*, Cell, 1998 92 735-745). The VEGF receptor was found to specifically bind the $VEGF_{165}$ isoform via the exon 7 encoded sequence, which shows weak affinity for heparin (Soker *et al.*, Cell, 1998 92 735-745). Surprisingly, the receptor was shown to be identical to human neuropilin-1 (NP-1), a receptor involved in early stage neuromorphogenesis. PlGF-2 also appears to interact with NP-1 (Migdal *et al.*, J. Biol. Chem., 1998 273 22272-22278).

VEGFR-1, VEGFR-2 and VEGFR-3 are expressed differently by endothelial cells. Both VEGFR-1 and VEGFR-2 are expressed in blood vessel endothelia (Oelrichs et al., Oncogene, 1992 8 11-18; Kaipainen et al., J. Exp. Med., 1993 178 2077-2088; Dumont et al., Dev. Dyn., 1995 203 80-92; Fong et al., Dev. Dyn., 1996 207 1-10) and VEGFR-3 is mostly expressed in the lymphatic endothelium of adult tissues (Kaipainen et al., Proc. Natl. Acad. Sci. USA, 1995 9 3566-3570). VEGFR-3 is also expressed in the blood vasculature surrounding tumors.

Disruption of the VEGFR genes results in aberrant development of the vasculature leading to embryonic lethality around midgestation. Analysis of embryos carrying a completely inactivated VEGFR-1 gene suggests that this receptor is required for functional organization of the endothelium (Fong et al., Nature, 1995 376 66-70). However, deletion of the intracellular tyrosine kinase domain of VEGFR-1 generates viable mice with a normal vasculature (Hiratsuka et al., Proc. Natl. Acad. Sci. USA 1998 95 9349-9354). The reasons underlying these differences remain to be explained but suggest that receptor signalling via the tyrosine kinase is not required for the proper function of VEGFR-1. Analysis of homozygous mice with inactivated alleles of VEGFR-2 suggests that this receptor is required for endothelial cell proliferation, hematopoesis and vasculogenesis (Shalaby et al., Nature, 1995 376 62-66; Shalaby et al., Cell, 1997 89 981-990). Inactivation of VEGFR-3 results in cardiovascular failure due to abnormal organization of the large vessels (Dumont et al. Science, 1998 282 946-949).

Although VEGFR-1 is mainly expressed in endothelial cells during development, it can also be found in hematopoetic precursor cells during early stages of embryogenesis (Fong et al., Nature, 1995 376 66-70). In adults, monocytes and

macrophages also express this receptor (Barleon et al., Blood, 1996 87 3336-3343). In embryos, VEGFR-1 is expressed by most, if not all, vessels (Breier et al., Dev. Dyn., 1995 204 228-239; Fong et al., Dev. Dyn., 1996 207 1-10).

5          The receptor VEGFR-3 is widely expressed on endothelial cells during early embryonic development but as embryogenesis proceeds becomes restricted to venous endothelium and then to the lymphatic endothelium (Kaipainen et al., Cancer Res., 1994 54 6571-6577; Kaipainen et al., Proc. Natl. Acad. Sci. 10     USA, 1995 92 3566-3570). VEGFR-3 is expressed on lymphatic endothelial cells in adult tissues. This receptor is essential for vascular development during embryogenesis. Targeted inactivation of both copies of the VEGFR-3 gene in mice resulted in defective blood vessel formation 15     characterized by abnormally organized large vessels with defective lumens, leading to fluid accumulation in the pericardial cavity and cardiovascular failure at post-coital day 9.5. On the basis of these findings it has been proposed that VEGFR-3 is required for the maturation of primary 20     vascular networks into larger blood vessels. However, the role of VEGFR-3 in the development of the lymphatic vasculature could not be studied in these mice because the embryos died before the lymphatic system emerged. Nevertheless it is assumed that VEGFR-3 plays a role in 25     development of the lymphatic vasculature and lymphangiogenesis given its specific expression in lymphatic endothelial cells during embryogenesis and adult life. This is supported by the finding that ectopic expression of VEGF-C, a ligand for VEGFR-3, in the skin of transgenic mice, resulted in lymphatic 30     endothelial cell proliferation and vessel enlargement in the dermis. Furthermore this suggests that VEGF-C may have a primary function in lymphatic endothelium, and a secondary

function in angiogenesis and permeability regulation which is
shared with VEGF (Joukov et al., EMBO J., 1996 15 290-298).

Some inhibitors of the VEGF/VEGF-receptor system have
been shown to prevent tumor growth via an anti-angiogenic
mechanism; see Kim et al., Nature, 1993 362 841-844 and Saleh
et al., Cancer Res., 1996 56 393-401.

As mentioned above, the VEGF family of growth factors
are members of the PDGF family.  PDGF plays a important role
in the growth and/or motility of connective tissue cells,
fibroblasts, myofibroblasts and glial cells (Heldin et al.,
"Structure of platelet-derived growth factor: Implications for
functional properties", Growth Factor, 1993 8 245-252).  In
adults, PDGF stimulates wound healing (Robson et al., Lancet,
1992 339 23-25).  Structurally, PDGF isoforms are disulfide-
bonded dimers of homologous A- and B-polypeptide chains,
arranged as homodimers (PDGF-AA and PDGF-BB) or a heterodimer
(PDGF-AB).

PDGF isoforms exert their effects on target cells by
binding to two structurally related receptor tyrosine kinases
(RTKs).  The alpha-receptor binds both the A- and B-chains of
PDGF, whereas the beta-receptor binds only the B-chain.  These
two receptors are expressed by many in vitro grown cell lines,
and are mainly expressed by mesenchymal cells in vivo.  The
PDGFs regulate cell proliferation, cell survival and
chemotaxis of many cell types in vitro (reviewed in Heldin et
al., Biochim Biophys Acta., 1998 1378 F79-113).  In vivo, they
exert their effects in a paracrine mode since they often are
expressed in epithelial (PDGF-A) or endothelial cells (PDGF-B)
in close apposition to the PDGFR expressing mesenchyme.  In
tumor cells and in cell lines grown in vitro, coexpression of
the PDGFs and the receptors generate autocrine loops which are
important for cellular transformation (Betsholtz et al., Cell,

1984 _39_ 447-57; Keating *et al.*, J. R. Coll Surg Edinb., 1990 _35_ 172-4). Overexpression of the PDGFs have been observed in several pathological conditions, including maligancies, arteriosclerosis, and fibroproliferative diseases (reviewed in Heldin *et al.*, The Molecular and Cellular Biology of Wound Repair, New York: Plenum Press, 1996, 249-273).

The importance of the PDGFs as regulators of cell proliferation and survival are well illustrated by recent gene targeting studies in mice that have shown distinct physiological roles for the PDGFs and their receptors despite the overlapping ligand specificities of the PDGFRs. Homozygous null mutations for either of the two PDGF ligands or the receptors are lethal. Approximately 50% of the homozygous PDGF-A deficient mice have an early lethal phenotype, while the surviving animals have a complex postnatal phenotype with lung emphysema due to improper alveolar septum formation because of a lack of alveolar myofibroblasts (Boström *et al.*, Cell, 1996 _85_ 863-873). The PDGF-A deficient mice also have a dermal phenotype characterized by thin dermis, misshapen hair follicles and thin hair (Karlsson *et al.*, Development, 1999 _126_ 2611-2). PDGF-A is also required for normal development of oligodendrocytes and subsequent myelination of the central nervous system (Fruttiger *et al.*, Development, 1999 _126_ 457-67). The phenotype of PDGFR-alpha deficient mice is more severe with early embryonic death at E10, incomplete cephalic closure, impaired neural crest development, cardiovascular defects, skeletal defects, and odemas [Soriano *et al.*, Development, 1997 _124_ 2691-70). The PDGF-B and PDGFR-beta deficient mice develop similar phenotypes that are characterized by renal, hematological and cardiovascular abnormalities (Levéen *et al.*, Genes Dev., 1994 _8_ 1875-1887; Soriano *et al.*, Genes Dev., 1994 _8_ 1888-96; Lindahl *et al.*,

Science, 1997 <u>277</u> 242-5; Lindahl, Development, 1998 <u>125</u>
3313-2), where the renal and cardiovascular defects, at least
in part, are due to the lack of proper recruitment of mural
cells (vascular smooth muscle cells, pericytes or mesangial
5       cells) to blood vessels (Levéen et al., Genes Dev., 1994 <u>8</u>
1875-1887; Lindahl et al., Science, 1997 <u>277</u> 242-5; Lindahl
et al., Development, 1998 <u>125</u> 3313-2).


10      <u>SUMMARY OF THE INVENTION</u>
        The invention generally provides an isolated novel growth
factor which has the ability to stimulate and/or enhance
proliferation or differentiation and/or growth and/or motility
of cells expressing a PDGF-C receptor including, but not
15      limited to, endothelial cells, connective tissue cells,
myofibroblasts and glial cells, an isolated polynucleotide
sequence encoding the novel growth factor, and compositions
useful for diagnostic and/or therapeutic applications.
        According to one aspect, the invention provides an
20      isolated and purified nucleic acid molecule which comprises
a polynucleotide sequence having at least 85% identity, more
preferably at least 90%, and most preferably at least 95%
identity to at least nucleotides 37-1071 of the sequence set
out in Figure 1 (SEQ ID NO:2), at least nucleotides 6-956 of
25      the sequence set out in Figure 3 (SEQ ID NO:3) or at least
nucleotides 196 to 1233 of the sequence set out in Figure 5
(SEQ ID NO:6). The sequence of at least nucleotides 37-1071
of the sequence set out in Figure 1 (SEQ ID NO:2) or at least
nucleotides 196 to 1233 of the sequence set out in Figure 5
30      (SEQ ID NO:6) encodes a novel polypeptide, designated PDGF-C
(formally designated "VEGF-F"), which is structurally
homologous to PDGF-A, PDGF-B, VEGF, VEGF-B, VEGF-C and VEGF-D.
In a preferred embodiment, the nucleic acid molecule is a cDNA

which comprises at least nucleotides 37-1071 of the sequence
set out in Figure 1 (SEQ ID NO:2), at least nucleotides 6-956
of the sequence set out in Figure 3 (SEQ ID NO:3) or at least
nucleotides 196 to 1233 of the sequence set out in Figure 5

5    (SEQ ID NO:6). This aspect of the invention also encompasses
DNA molecules having a sequence such that they hybridize under
stringent conditions with at least nucleotides 37-1071 of the
sequence set out in Figure 1 (SEQ ID NO:2), at least
nucleotides 6-956 of the sequence set out in Figure 3 (SEQ ID

10   NO:3) or at least nucleotides 196 to 1233 of the sequence set
out in Figure 5 (SEQ ID NO:6) or fragments thereof.

According to a second aspect, the polypeptide of the
invention has the ability to stimulate and/or enhance
proliferation and/or differentiation and/or growth and/or

15   motility of cells expressing a PDGF-C receptor including, but
not limited to, endothelial cells, connective tissue cells,
myofibroblasts and glial cells and comprises a sequence of
amino acids corresponding to the amino acid sequence set out
in Figure 2 (SEQ ID NO:3), Figure 4 (SEQ ID NO:5) or Figure

20   6 (SEQ ID NO:7), or a fragment or analog thereof which has the
ability to stimulate and/or enhance proliferation and/or
differentiation and/or growth and/or motility of cells
expressing a PDGF-C receptor including, but not limited to,
endothelial cells, connective tissue cells (such as

25   fibroblasts), myofibroblasts and glial cells. Preferably the
polypeptides have at least 85% identity, more preferably at
least 90%, and most preferably at least 95% identity to the
amino acid sequence of in Figure 2 (SEQ ID NO:3), Figure 4
(SEQ ID NO:5) or Figure 6 (SEQ ID NO:7), or a fragment or

30   analog thereof having the biological activity of PDGF-C. A
preferred fragment is a truncated form of PDGF-C comprising
a portion of the PDGF/VEGF homology domain (PVHD) of PDGF-C.
The minimal domain is residues 230-345. However, the domain

can extend towards the N terminus up to residue 164. Herein the PVHD is defined as truncated PDGF-C. The truncated PDGF-C is an activated form of PDGF-C.

As used in this application, percent sequence identity is determined by using the alignment tool of "MEGALIGN" from the Lasergene package (DNASTAR, Ltd. Abacus House, Manor Road, West Ealing, London W130AS United Kingdom) and using its preset conditions. The alignment is then refined manually, and the number of identities are estimated in the regions available for a comparison.

Preferably the polypeptide or the encoded polypeptide from a polynucleotide has the ability to stimulate one or more of proliferation, differentiation, motility, survival or vascular permeability of cells expressing a PDGF-C receptor including, but not limited to, vascular endothelial cells, lymphatic endothelial cells, connective tissue cells (such as fibroblasts), myofibroblasts and glial cells. Preferably the polypeptide or the encoded polypeptide from a polynucleotide has the ability to stimulate wound healing. PDGF-C can also have antagonistic effects on cells, but are included in the biological activities of PDGF-C. These abilities are referred to hereinafter as "biological activities of PDGF-C" and can be readily tested by methods known in the art.

As used herein, the term "PDGF-C" collectively refers to the polypeptides of Figure 2 (SEQ ID NO:3), Figure 4 (SEQ ID NO:5) or Figure 6 (SEQ ID NO:7), and fragments or analogs thereof which have the biological activity of PDGF-C as defined above, and to a polynucleotide which can code for PDGF-C, or a fragment or analog thereof having the biological activity of PDGF-C. The polynucleotide can be naked and/or in a vector or liposome.

In another preferred aspect, the invention provides a polypeptide possessing an amino acid sequence:

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PXCLLVXRCGGXCXCC    (SEQ ID NO:1)

which is unique to PDGF-C and differs from the other members
of the PDGF/VEGF family of growth factors because of the
insertion of the three amino acid residues (NCA) between the
5    third and fourth cysteines (see Figure 9 - SEQ ID NOs:8-17).

Polypeptides comprising conservative substitutions,
insertions, or deletions, but which still retain the
biological activity of PDGF-C are clearly to be understood to
be within the scope of the invention. Persons skilled in the
10   art will be well aware of methods which can readily be used
to generate such polypeptides, for example the use of site-
directed mutagenesis, or specific enzymatic cleavage and
ligation. The skilled person will also be aware that
peptidomimetic compounds or compounds in which one or more
15   amino acid residues are replaced by a non-naturally occurring
amino acid or an amino acid analog may retain the required
aspects of the biological activity of PDGF-C. Such compounds
can readily be made and tested by methods known in the art,
and are also within the scope of the invention.

20   In addition, possible variant forms of the PDGF-C
polypeptide which may result from alternative splicing, as are
known to occur with VEGF and VEGF-B, and naturally-occurring
allelic variants of the nucleic acid sequence encoding PDGF-C
are encompassed within the scope of the invention. Allelic
25   variants are well known in the art, and represent alternative
forms or a nucleic acid sequence which comprise substitution,
deletion or addition of one or more nucleotides, but which do
not result in any substantial functional alteration of the
encoded polypeptide.

30   Such variant forms of PDGF-C can be prepared by targeting
non-essential regions of the PDGF-C polypeptide for
modification. These non-essential regions are expected to
fall outside the strongly-conserved regions indicated in

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Figure 9 (SEQ ID NOs:8-17). In particular, the growth factors of the PDGF family, including VEGF, are dimeric, and VEGF, VEGF-B, VEGF-C, VEGF-D, PlGF, PDGF-A and PDGF-B show complete conservation of eight cysteine residues in the N-terminal domains, i.e. the PDGF/VEGF-like domains (Olofsson et al., Proc. Natl. Acad. Sci. USA, 1996 93 2576-2581; Joukov et al., EMBO J., 1996 15 290-298). These cysteines are thought to be involved in intra- and inter-molecular disulfide bonding. In addition there are further strongly, but not completely, conserved cysteine residues in the C-terminal domains. Loops 1, 2 and 3 of each subunit, which are formed by intra-molecular disulfide bonding, are involved in binding to the receptors for the PDGF/VEGF family of growth factors (Andersson et al., Growth Factors, 1995 12 159-164).

Persons skilled in the art thus are well aware that these cysteine residues should be preserved in any proposed variant form, and that the active sites present in loops 1, 2 and 3 also should be preserved. However, other regions of the molecule can be expected to be of lesser importance for biological function, and therefore offer suitable targets for modification. Modified polypeptides can readily be tested for their ability to show the biological activity of PDGF-C by routine activity assay procedures such as the fibroblast proliferation assay of Example 6.

It is contemplated that some modified PDGF-C polypeptides will have the ability to bind to PDGF-C receptors on cells including, but not limited to, endothelial cells, connective tissue cells, myofibroblasts and/or glial cells, but will be unable to stimulate cell proliferation, differentiation, migration, motility or survival or to induce vascular proliferation, connective tissue development or wound healing. These modified polypeptides are expected to be able to act as competitive or non-competitive inhibitors of the PDGF-C

polypeptides and growth factors of the PDGF/VEGF family, and
to be useful in situations where prevention or reduction of
the PDGF-C polypeptide or PDGF/VEGF family growth factor
action is desirable. Thus such receptor-binding but non-
5      mitogenic, non-differentiation inducing, non-migration
inducing, non-motility inducing, non-survival promoting, non-
connective tissue development promoting, non-wound healing or
non-vascular proliferation inducing variants of the PDGF-C
polypeptide are also within the scope of the invention, and
10     are referred to herein as "receptor-binding but otherwise
inactive variant". Because PDGF-C forms a dimer in order to
activate its only known receptor, it is contemplated that one
monomer comprises the receptor-binding but otherwise inactive
variant modified PDGF-C polypeptide and a second monomer
15     comprises a wild-type PDGF-C or a wild-type growth factor of
the PDGF/VEGF family. These dimers can bind to its
corresponding receptor but cannot induce downstream signaling.

        It is also contemplated that there are other modified
PDGF-C polypeptides that can prevent binding of a wild-type
20     PDGF-C or a wild-type growth factor of the PDGF/VEGF family
to its corresponding receptor on cells including, but not
limited to, endothelial cells, connective tissue cells (such
as fibroblasts), myofibroblasts and/or glial cells. Thus
these dimers will be unable to stimulate endothelial cell
25     proliferation, differentiation, migration, survival, or induce
vascular permeability, and/or stimulate proliferation and/or
differentiation and/or motility of connective tissue cells,
myofibroblasts or glial cells. These modified polypeptides
are expected to be able to act as competitive or non-
30     competitive inhibitors of the PDGF-C growth factor or a growth
factor of the PDGF/VEGF family, and to be useful in situations
where prevention or reduction of the PDGF-C growth factor or
PDGF/VEGF family growth factor action is desirable. Such

situations include the tissue remodeling that takes place during invasion of tumor cells into a normal cell population by primary or metastatic tumor formation. Thus such the PDGF-C or PDGF/VEGF family growth factor-binding but non-mitogenic,

5   non-differentiation inducing, non-migration inducing, non-motility inducing, non-survival promoting, non-connective tissue promoting, non-wound healing or non-vascular proliferation inducing variants of the PDGF-C growth factor are also within the scope of the invention, and are referred

10  to herein as "the PDGF-C growth factor-dimer forming but otherwise inactive or interfering variants".

An example of a PDGF-C growth factor-dimer forming but otherwise inactive or interfering variant is where the PDGF-C has a mutation which prevents cleavage of CUB domain from the

15  protein. It is further contemplated that a PDGF-C growth factor-dimer forming but otherwise inactive or interfering variant could be made to comprise a monomer, preferably an activated monomer, of VEGF, VEGF-B, VEGF-C, VEGF-D, PDGF-C, PDGF-A, PDGF-B or PlGF linked to a CUB domain that has a

20  mutation which prevents cleavage of CUB domain from the protein. Dimers formed with the above mentioned PDGF-C growth factor-dimer forming but otherwise inactive or interfering variants and the monomers linked to the mutant CUB domain would be unable to bind to their corresponding receptors.

25  A variation on this contemplation would be to insert a proteolytic site between an activated monomer of VEGF, VEGF-B, VEGF-C, VEGF-D, PDGF-C, PDGF-A, PDGF-B or PlGF and the mutant CUB domain linkage which is dimerized to an activated monomer of VEGF, VEGF-B, VEGF-C, VEGF-D, PDGF-C, PDGF-A, PDGF-B or

30  PlGF. An addition of the specific protease(s) for this proteolytic site would cleave the CUB domain and thereby release an activated dimer that can then bind to its

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corresponding receptor.  In this way, a controlled release of
an activated dimer is made possible.

According to a third aspect, the invention provides a
purified and isolated nucleic acid encoding a polypeptide or
polypeptide fragment of the invention as defined above.  The
nucleic acid may be DNA, genomic DNA, cDNA or RNA, and may be
single-stranded or double stranded.  The nucleic acid may be
isolated from a cell or tissue source, or of recombinant or
synthetic origin.  Because of the degeneracy of the genetic
code, the person skilled in the art will appreciate that many
such coding sequences are possible, where each sequence
encodes the amino acid sequence shown in Figure 2 (SEQ ID
NO:3), Figure 4 (SEQ ID NO:5) or Figure 6 (SEQ ID NO:7), a
bioactive fragment or analog thereof, a receptor-binding but
otherwise inactive or partially inactive variant thereof or
a PDGF-C-dimer forming but otherwise inactive or interfering
variants thereof.

A fourth aspect of the invention provides vectors
comprising the cDNA of the invention or a nucleic acid
molecule according to the third aspect of the invention, and
host cells transformed or transfected with nucleic acids
molecules or vectors of the invention.   These may be
eukaryotic or prokaryotic in origin.   These cells are
particularly suitable for expression of the polypeptide of the
invention, and include insect cells such as Sf9 cells,
obtainable from the American Type Culture Collection
(ATCC SRL-171), transformed with a baculovirus vector, and the
human embryo kidney cell line 293-EBNA transfected by a
suitable expression plasmid.   Preferred vectors of the
invention are expression vectors in which a nucleic acid
according to the invention is operatively connected to one or
more appropriate promoters and/or other control sequences,
such that appropriate host cells transformed or transfected

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with the vectors are capable of expressing the polypeptide of
the invention.  Other preferred vectors are those suitable for
transfection of mammalian cells, or for gene therapy, such as
adenoviral-,    vaccinia-    or    retroviral-based    vectors    or

5       liposomes.  A variety of such vectors is known in the art.

The invention also provides a method of making a vector
capable of expressing a polypeptide encoded by a nucleic acid
according    to    the    invention,    comprising    the    steps    of
operatively   connecting   the   nucleic   acid   to   one   or   more

10      appropriate   promoters   and/or   other   control   sequences,   as
described above.

The   invention   further   provides   a   method   of   making   a
polypeptide according to the invention, comprising the steps
of expressing a nucleic acid or vector of the invention in a

15      host cell, and isolating the polypeptide from the host cell
or from the host cell's growth medium.

In   a   further   aspect,   the   invention   provides   an
antibody   specifically   reactive   with   a   polypeptide   of   the
invention or a fragment of the polypeptide.  This aspect of

20      the   invention   includes   antibodies   specific   for   the   variant
forms,   immunoreactive   fragments,   analogs   and   recombinants   of
PDGF-C.  Such antibodies are useful as inhibitors or agonists
of   PDGF-C   and   as   diagnostic   agents   for   detecting   and
quantifying PDGF-C.  Polyclonal or monoclonal antibodies may

25      be used.  Monoclonal and polyclonal antibodies can be raised
against polypeptides of the invention or fragment or analog
thereof using standard methods in the art.  In addition the
polypeptide can be linked to an epitope tag, such as the FLAG®
octapeptide (Sigma, St. Louis, MO), to assist in affinity

30      purification.    For    some    purposes,    for    example    where    a
monoclonal antibody is to be used to inhibit effects of PDGF-C
in a clinical situation, it may be desirable to use humanized
or   chimeric   monoclonal   antibodies.    Such   antibodies   may   be

further modified by addition of cytotoxic or cytostatic drugs. Methods for producing these, including recombinant DNA methods, are also well known in the art.

This aspect of the invention also includes an antibody
5      which recognizes PDGF-C and is suitably labeled.

Polypeptides or antibodies according to the invention may be labeled with a detectable label, and utilized for diagnostic purposes. Similarly, the thus-labeled polypeptide of the invention may be used to identify its corresponding
10     receptor *in situ*. The polypeptide or antibody may be covalently or non-covalently coupled to a suitable supermagnetic, paramagnetic, electron dense, ecogenic or radioactive agent for imaging. For use in diagnostic assays, radioactive or non-radioactive labels may be used. Examples
15     of radioactive labels include a radioactive atom or group, such as $^{125}$I or $^{32}$P. Examples of non-radioactive labels include enzymatic labels, such as horseradish peroxidase or fluorimetric labels, such as fluorescein-5-isothiocyanate (FITC). Labeling may be direct or indirect, covalent or non-
20     covalent.

Clinical applications of the invention include diagnostic applications, acceleration of angiogenesis in tissue or organ transplantation, or stimulation of wound healing, or connective tissue development, or to establish collateral
25     circulation in tissue infarction or arterial stenosis, such as coronary artery disease, and inhibition of angiogenesis in the treatment of cancer or of diabetic retinopathy and inhibition of tissue remodeling that takes place during invasion of tumor cells into a normal cell population by
30     primary or metastatic tumor formation. Quantitation of PDGF-C in cancer biopsy specimens may be useful as an indicator of future metastatic risk.

PDGF-C may also be relevant to a variety of lung

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conditions. PDGF-C assays could be used in the diagnosis of various lung disorders. PDGF-C could also be used in the treatment of lung disorders to improve blood circulation in the lung and/or gaseous exchange between the lungs and the blood stream. Similarly, PDGF-C could be used to improve blood circulation to the heart and $O_2$ gas permeability in cases of cardiac insufficiency. In a like manner, PDGF-C could be used to improve blood flow and gaseous exchange in chronic obstructive airway diseases.

Thus the invention provides a method of stimulation of angiogenesis, lymphangiogenesis, neovascularization, connective tissue development and/or wound healing in a mammal in need of such treatment, comprising the step of administering an effective dose of PDGF-C, or a fragment or an analog thereof which has the biological activity of PDGF-C to the mammal. Optionally the PDGF-C, or fragment or analog thereof may be administered together with, or in conjunction with, one or more of VEGF, VEGF-B, VEGF-C, VEGF-D, PlGF, PDGF-A, PDGF-B, FGF and/or heparin.

Conversely, PDGF-C antagonists (e.g. antibodies and/or competitive or noncompetitive inhibitors of binding of PDGF-C in both dimer formation and receptor binding) could be used to treat conditions, such as congestive heart failure, involving accumulation of fluid in, for example, the lung resulting from increases in vascular permeability, by exerting an offsetting effect on vascular permeability in order to counteract the fluid accumulation. PDGF-C can also be used to treat fibrotic conditions including those found in the lung, kidney and liver. Administrations of PDGF-C could be used to treat malabsorptive syndromes in the intestinal tract, liver or kidneys as a result of its blood circulation increasing and vascular permeability increasing activities.

Thus, the invention provides a method of inhibiting
angiogenesis, lymphangiogenesis, neovascularization,
connective tissue development and/or wound healing in a mammal
in need of such treatment, comprising the step of
5    administering an effective amount of an antagonist of PDGF-C
to the mammal. The antagonist may be any agent that prevents
the action of PDGF-C, either by preventing the binding of
PDGF-C to its corresponding receptor on the target cell, or
by preventing activation of the receptor, such as using
10   receptor-binding PDGF-C variants. Suitable antagonists
include, but are not limited to, antibodies directed against
PDGF-C; competitive or non-competitive inhibitors of binding
of PDGF-C to the PDGF-C receptor(s), such as the receptor-
binding or PDGF-C dimer-forming but non-mitogenic PDGF-C
15   variants referred to above; compounds that bind to PDGF-C
and/or modify or antagonize its function, and anti-sense
nucleotide sequences as described below.

A method is provided for determining agents that bind to
an activated truncated form of PDGF-C. The method comprises
20   contacting an activated truncated form of PDGF-C with a test
agent and monitoring binding by any suitable means. Agents
can include both compounds and other proteins.

The invention provides a screening system for discovering
agents that bind an activated truncated form of PDGF-C. The
25   screening system comprises preparing an activated truncated
form of PDGF-C, exposing the activated truncated form of PDGF-
C to a test agent, and quantifying the binding of said agent
to the activated truncated form of PDGF-C by any suitable
means. This screening system can also be used to identify
30   agents which inhibit the proteolytic cleavage of the full
length PDGF-C protein and thereby prevent the release of the
activated truncated form of PDGF-C. For this use, the full
length PDGF-C must be prepared.

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Use of this screen system provides a means to determine compounds that may alter the biological function of PDGF-C. This screening method may be adapted to large-scale, automated procedures such as a PANDEX® (Baxter-Dade Diagnostics) system,

5      allowing for efficient high-volume screening of potential therapeutic agents.

For this screening system, an activated truncated form of PDGF-C or full length PDGF-C is prepared as described herein, preferably using recombinant DNA technology. A test

10     agent, e.g. a compound or protein, is introduced into a reaction vessel containing the activated truncated form of or full length PDGF-C. Binding of the test agent to the activated truncated form of or full length PDGF-C is determined by any suitable means which include, but is not

15     limited to, radioactively- or chemically-labeling the test agent. Binding of the activated truncated form of or full length PDGF-C may also be carried out by a method disclosed in U.S. Patent 5,585,277, which is incorporated by reference. In this method, binding of the test agent to the activated

20     truncated form of or full length PDGF-C is assessed by monitoring the ratio of folded protein to unfolded protein. Examples of this monitoring can include, but are not limited to, monitoring the sensitivity of the activated truncated form of or full length PDGF-C to a protease, or amenability to

25     binding of the protein by a specific antibody against the folded state of the protein.

Those of skill in the art will recognize that $IC_{50}$ values are dependent on the selectivity of the agent tested. For example, an agent with an $IC_{50}$ which is less than 10 nM is

30     generally considered an excellent candidate for drug therapy. However, an agent which has a lower affinity, but is selective for a particular target, may be an even better candidate. Those skilled in the art will recognize that any information

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regarding the binding potential, inhibitory activity or selectivity of a particular agent is useful toward the development of pharmaceutical products.

Where a PDGF-C or a PDGF-C antagonist is to be used for therapeutic purposes, the dose(s) and route of administration will depend upon the nature of the patient and condition to be treated, and will be at the discretion of the attending physician or veterinarian. Suitable routes include oral, subcutaneous, intramuscular, intraperitoneal or intravenous injection, parenteral, topical application, implants *etc.* Topical application of PDGF-C may be used in a manner analogous to VEGF. For example, where used for wound healing or other use in which enhanced angiogenesis is advantageous, an effective amount of the truncated active form of PDGF-C is administered to an organism in need thereof in a dose between about 0.1 and 1000 µg/kg body weight.

The PDGF-C or a PDGF-C antagonist may be employed in combination with a suitable pharmaceutical carrier. The resulting compositions comprise a therapeutically effective amount of PDGF-C or a PDGF-C antagonist, and a pharmaceutically acceptable non-toxic salt thereof, and a pharmaceutically acceptable solid or liquid carrier or adjuvant. Examples of such a carrier or adjuvant include, but are not limited to, saline, buffered saline, Ringer's solution, mineral oil, talc, corn starch, gelatin, lactose, sucrose, microcrystalline cellulose, kaolin, mannitol, dicalcium phosphate, sodium chloride, alginic acid, dextrose, water, glycerol, ethanol, thickeners, stabilizers, suspending agents and combinations thereof. Such compositions may be in the form of solutions, suspensions, tablets, capsules, creams, salves, elixirs, syrups, wafers, ointments or other conventional forms. The formulation to suit the mode of administration. Compositions which comprise PDGF-C may

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optionally further comprise one or more of PDGF-A, PDGF-B, VEGF, VEGF-B, VEGF-C, VEGF-D, PlGF and/or heparin. Compositions comprising PDGF-C will contain from about 0.1% to 90% by weight of the active compound(s), and most generally from about 10% to 30%.

For intramuscular preparations, a sterile formulation, preferably a suitable soluble salt form of the truncated active form of PDGF-C, such as hydrochloride salt, can be dissolved and administered in a pharmaceutical diluent such as pyrogen-free water (distilled), physiological saline or 5% glucose solution. A suitable insoluble form of the compound may be prepared and administered as a suspension in an aqueous base or a pharmaceutically acceptable oil base, e.g. an ester of a long chain fatty acid such as ethyl oleate.

According to yet a further aspect, the invention provides diagnostic/prognostic devices typically in the form of test kits. For example, in one embodiment of the invention there is provided a diagnostic/prognostic test kit comprising an antibody to PDGF-C and a means for detecting, and more preferably evaluating, binding between the antibody and PDGF-C. In one preferred embodiment of the diagnostic/prognostic device according to the invention, a second antibody (the secondary antibody) directed against antibodies of the same isotype and animal source of the antibody directed against PDGF-C (the primary antibody) is provided. The secondary antibody is coupled to a detectable label, and then either an unlabeled primary antibody or PDGF-C is substrate-bound so that the PDGF-C/primary antibody interaction can be established by determining the amount of label bound to the substrate following binding between the primary antibody and PDGF-C and the subsequent binding of the labeled secondary antibody to the primary antibody. In a particularly preferred embodiment of the invention, the diagnostic/prognostic device

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may be provided as a conventional enzyme-linked immunosorbent assay (ELISA) kit.

In another alternative embodiment, a diagnostic/prognostic device may comprise polymerase chain reaction means for establishing sequence differences of a PDGF-C of a test individual and comparing this sequence structure with that disclosed in this application in order to detect any abnormalities, with a view to establishing whether any aberrations in PDGF-C expression are related to a given disease condition.

In addition, a diagnostic/prognostic device may comprise a restriction length polymorphism (RFLP) generating means utilizing restriction enzymes and genomic DNA from a test individual to generate a pattern of DNA bands on a gel and comparing this pattern with that disclosed in this application in order to detect any abnormalities, with a view to establishing whether any aberrations in PDGF-C expression are related to a given disease condition.

In accordance with a further aspect, the invention relates to a method of detecting aberrations in PDGF-C gene in a test subject which may be associated with a disease condition in the test subject. This method comprises providing a DNA or RNA sample from said test subject; contacting the DNA sample or RNA with a set of primers specific to PDGF-C DNA operatively coupled to a polymerase and selectively amplifying PDGF-C DNA from the sample by polymerase chain reaction, and comparing the nucleotide sequence of the amplified PDGF-C DNA from the sample with the nucleotide sequences shown in Figure 1 (SEQ ID NO:2) or Figure 3 (SEQ ID NO:5). The invention also includes the provision of a test kit comprising a pair of primers specific to PDGF-C DNA operatively coupled to a polymerase, whereby said

polymerase is enabled to selectively amplify PDGF-C DNA from a DNA sample.

The invention also provides a method of detecting PDGF-C in a biological sample, comprising the step of contacting the
5      sample with a reagent capable of binding PDGF-C, and detecting the binding.  Preferably the reagent capable of binding PDGF-C is an antibody directed against PDGF-C, particularly preferably a monoclonal antibody.  In a preferred embodiment the binding and/or extent of binding is detected by means of
10     a detectable label; suitable labels are discussed above.

In another aspect, the invention relates to a protein dimer comprising the PDGF-C polypeptide, particularly a disulfide-linked dimer.  The protein dimers of the invention include both homodimers of PDGF-C polypeptide and heterodimers
15     of PDGF-C and VEGF, VEGF-B, VEGF-C, VEGF-D, PlGF, PDGF-A or PDGF-B.

According to a yet further aspect of the invention there is provided a method for isolation of PDGF-C comprising the step of exposing a cell which expresses PDGF-C to heparin to
20     facilitate release of PDGF-C from the cell, and purifying the thus-released PDGF-C.

Another aspect of the invention involves providing a vector comprising an anti-sense nucleotide sequence which is complementary to at least a part of a DNA sequence which
25     encodes PDGF-C or a fragment or analog thereof that has the biological activity of PDGF-C.  In addition the anti-sense nucleotide sequence can be to the promoter region of the PDGF-C gene or other non-coding region of the gene which may be used to inhibit, or at least mitigate, PDGF-C expression.
30     According to a yet further aspect of the invention such a vector comprising an anti-sense sequence may be used to inhibit, or at least mitigate, PDGF-C expression.  The use of a vector of this type to inhibit PDGF-C expression is favored

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in instances where PDGF-C expression is associated with a
disease, for example where tumors produce PDGF-C in order to
provide for angiogenesis, or tissue remodeling that takes
place during invasion of tumor cells into a normal cell

5      population by primary or metastatic tumor formation.
Transformation of such tumor cells with a vector containing
an anti-sense nucleotide sequence would inhibit or retard
growth of the tumor or tissue remodeling.

       Another aspect of the invention relates to the discovery

10     that the full length PDGF-C protein is likely to be a latent
growth factor that needs to be activated by proteolytic
processing to release an active PDGF/VEGF homology domain.
A putative proteolytic site is found in residues 231-234 in
the full length protein, residues -RKSR-. This is a dibasic

15     motif. This site is structurally conserved in the mouse PDGF-
C.   The -RKSR- putative proteolytic site is also found in
PDGF-A, PDGF-B, VEGF-C and VEGF-D. In these four proteins,
the putative proteolytic site is also found just before the
minimal domain for the PDGF/VEGF homology domain. Together

20     these facts indicate that this is the proteolytic site.

       Preferred proteases include, but are not limited, to
plasmin, Factor X and enterokinase. The N-terminal CUB domain
may function as an inhibitory domain which might be used to
keep PDGF-C in a latent form in some extracellular compartment

25     and which is removed by limited proteolysis when PDGF-C is
needed.

       According to this aspect of the invention, a method is
provided for producing an activated truncated form of PDGF-C
or for regulating receptor-binding specificity of PDGF-C.

30     These methods comprise the steps of expressing an expression
vector comprising a polynucleotide encoding a polypeptide
having the biological activity of PDGF-C and supplying a
proteolytic amount of at least one enzyme for processing the

expressed polypeptide to generate the activated truncated form of PDGF-C.

This aspect also includes a method for selectively activating a polypeptide having a growth factor activity. This method comprises the step expressing an expression vector comprising a polynucleotide encoding a polypeptide having a growth factor activity, a CUB domain and a proteolytic site between the polypeptide and the CUB domain, and supplying a proteolytic amount of at least one enzyme for processing the expressed polypeptide to generate the activated polypeptide having a growth factor activity.

In addition, this aspect includes the isolation of a nucleic acid molecule which codes for a polypeptide having the biological activity of PDGF-C and a polypeptide thereof which comprises a proteolytic site having the amino acid sequence RKSR or a structurally conserved amino acid sequence thereof.

Also this aspect includes an isolated dimer comprising an activated monomer of PDGF-C and an activated monomer of VEGF, VEGF-B, VEGF-C, VEGF-D, PDGF-C, PDGF-A, PDGF-B or P1GF linked to a CUB domain, or alternatively, an activated monomer of VEGF, VEGF-B, VEGF-C, VEGF-D, PDGF-C, PDGF-A, PDGF-B or P1GF and an activated monomer of PDGF-C linked to a CUB domain. The isolated dimer may or may not include a proteolytic site between the activator monomer and the CUB domain linkage.

Polynucleotides of the invention such as those described above, fragments of those polynucleotides, and variants of those polynucleotides with sufficient similarity to the non-coding strand of those polynucleotides to hybridize thereto under stringent conditions all are useful for identifying, purifying, and isolating polynucleotides encoding other, non-human, mammalian forms of PDGF-C. Thus, such polynucleotide fragments and variants are intended as aspects of the

invention. Exemplary stringent hybridization conditions are
as follows: hybridization at 42°C in 5X SSC, 20 mM NaPO$_4$,
pH 6.8, 50% formamide; and washing at 42°C in 0.2X SSC. Those
skilled in the art understand that it is desirable to vary

5     these conditions empirically based on the length and the GC
nucleotide base content of the sequences to be hybridized, and
that formulas for determining such variation exist. See for
example Sambrook *et al*, "Molecular Cloning: A Laboratory
Manual", Second Edition, pages 9.47-9.51, Cold Spring Harbor,

10    New York: Cold Spring Harbor Laboratory (1989).

Moreover, purified and isolated polynucleotides encoding
other, non-human, mammalian PDGF-C forms also are aspects of
the invention, as are the polypeptides encoded thereby and
antibodies that are specifically immunoreactive with the non-

15    human PDGF-C variants. Thus, the invention includes a
purified and isolated mammalian PDGF-C polypeptide and also
a purified and isolated polynucleotide encoding such a
polypeptide.

It will be clearly understood that nucleic acids and

20    polypeptides of the invention may be prepared by synthetic
means or by recombinant means, or may be purified from natural
sources.

It will be clearly understood that for the purposes of
this specification the word "comprising" means "included but

25    not limited to". The corresponding meaning applies to the
word "comprises".


BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 (SEQ ID NO:2) shows the complete nucleotide

30    sequence of cDNA encoding a human PDGF-C (hPDGF-C)(2108 bp);

Figure 2 (SEQ ID NO:3) shows the deduced amino acid
sequence of full-length hPDGF-C which consists of 345 amino

acid residues (the translated part of the cDNA corresponds to nucleotides 37 to 1071 of Figure 1);

Figure 3 (SEQ ID NO:4) shows a cDNA sequence encoding a fragment of human PDGF-C (hPDGF-C) (1536 bp);

5     Figure 4 (SEQ ID NO:5) shows a deduced amino acid sequence of a fragment of hPDGF-C (translation of nucleotides 3 to 956 of the nucleotide sequence of Figure 3);

Figure 5 (SEQ ID NO:6) shows a nucleotide sequence of a murine PDGF-C (mPDGF-C) cDNA;

10    Figure 6 (SEQ ID NO:7) shows the deduced amino acid sequence of a fragment of mPDGF-C (the translated part of the cDNA corresponds to nucleotides 196 to 1233 of Figure 5);

Figure 7 shows a comparative sequence alignment of the hPDGF-C amino acid sequence of Figure 2 (SEQ ID NO:3) with the
15    mPDGF-C amino acid sequence of Figure 6 (SEQ ID NO:7);

Figure 8 shows a schematic structure of mPDGF-C with a signal sequence (striped box), a N-terminal C1r/C1s/embryonic sea urchin protein Uegf/bone morphogenetic protein 1 (CUB) domain and the C-terminal PDGF/VEGF-homology domain (open
20    boxes);

Figure 9 shows a comparative sequence alignment of the PDGF/VEGF-homology domains in human and mouse PDGF-C with other members of the VEGF/PDGF family of growth factors (SEQ ID NOs:8-17, respectively);

25    Figure 10 shows a phylogenetic tree of several growth factors belonging to the VEGF/PDGF family;

Figure 11 provides the amino acid sequence alignment of the CUB domain present in human and mouse PDGF-Cs (SEQ ID NOs:18 and 19, respectively) and other CUB domains present in
30    human bone morphogenic protein-1 (hBMP-1, 3 CUB domains CUB1-3) (SEQ ID NOs:20-22, respectively) and in human neuropilin-1 (2 CUB domains) (SEQ ID NOs:23 and 24, respectively);

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Figure 12 shows a Northern blot analysis of the expression of PDGF-C transcripts in several human tissues;

Figure 13 shows the regulation of PDGF-C mRNA expression by hypoxia; and

5      Figure 14 shows the expression of PDGF-C in human tumor cell lines.

Figure 15 shows the results of immunoblot detection of full length human PDGF-C in transfected COS-1 cells.

Figure 16 shows isolation and partial characterization 10      of full length PDGF-C.

Figure 17 shows isolation and partial characterization of a truncated form of human PDGF-C containing the PDGF/VEGF homology domain only.

Figure 18 provides a standard curve for the binding of 15      labeled PDGF-BB homodimers to PAE-1 cells expressing PDGF alpha receptor.

Figure 19 provides a graphic representation of the inhibition of binding of labeled PDGF-BB to PAE-1 cells expressing PDGF alpha receptor by increasing amounts of 20      purified full length and truncated PDGF-CC proteins.

Figure 20 shows the effects of the full length and truncated PDGF-CC homodimers on the phosphorylation of PDGF alpha-receptor.

Figure 21 shows the mitogenic activities of the full 25      length and truncated PDGF-CC homodimers on fibroblasts.

Figure 22 graphically presents the results of the binding assay of truncated PDGF-C to the PDGF receptors.

Figure 23 shows the immunoblot of the undigested full length PDGF-C protein and the plasmin-generated 26-28 kDa 30      species.

Figure 24 graphically presents the results of the competitive binding assay of full-length PDGF-C and truncated PDGF-C for PDGFR-alpha receptors.

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Figure 25 shows the analyses by SDS-PAGE of the human PDGF-C CUB domain under reducing and non-reducing conditions.

Figures 26A-26V show PDGF-C expression in the developing mouse embryo.

5      Figures 27A-27F show PDGF-C, PDGF-A and PDGFR-alpha expression in the developing kidney.

Figures 28A-28F show histology of E 16.5 kidneys from wildtype (Figures 28A and 28C), PDGFR-alpha -/- (Figures 28B and 28F, PDGF-A -/- (Figure 28D) and PDGF-A/PDGF-B double -/-

10     (Figure 28E) kidneys.


DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Figure 1 (SEQ ID NO:2) shows the complete nucleotide

15     sequence of cDNA encoding a human PDGF-C (hPDGF-C) (2108 bp), which is a new member of the VEGF/PDGF family. A clone #4 (see Figures 3 and 4 - SEQ ID NOs:4 and 5) encoding hPDGF-C was not full length and lacked approximately 80 base pairs of coding sequence when compared to the mouse protein

20     (corresponding to 27 amino acids). Additional cDNA clones were isolated from a human fetal lung cDNA library to obtain an insert which included this missing sequence. Clone #10 had a longer insert than clone #4. The insert of clone #10 was sequenced in the 5' region and it was found to contain the

25     missing sequence. Clone #10 was found to include the full sequence of human PDGF-C. Some 5'-untranslated sequence, the translated part of the cDNA encoding human PDGF-C and some 3'-untranslated nucleotide sequence are shown in Figure 1 (SEQ ID NO:2). A stop codon in frame is located 21 bp upstream of

30     the initiation ATG (the initiation ATG is underlined in Figure 1).

Work to isolate this new human PDGF/VEGF began after a search of the expressed sequence tag (EST) database, dbEST,

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at the National Center for Biotechnology Information (NCBI)
in Washington, DC, identified a human EST sequence (W21436)
which appears to encode part of the human homolog of the mouse
PDGF-C. Based on the human EST sequence, two oligonucleotides
5    were designed:

        5'-GAA GTT GAG GAA CCC AGT G-3' forward (SEQ ID NO:25)

        5'-CTT GCC AAG AAG TTG CCA AG-3' reverse (SEQ ID NO:26).
These oligonucleotides were used to amplify by polymerase
chain reaction (PCR) a polynucleotide of 348 bps from a Human
10   Fetal Lung 5'-STRETCH PLUS λgt10 cDNA library, which was
obtained commercially from Clontech. The PCR product was
cloned into the pCR 2.1-vector of the Original TA Cloning Kit
(Invitrogen). Subsequently, the 348 bps cloned PCR product
was used to construct a hPDGF-C probe according to standard
15   techniques.

        $10^6$ lambda-clones of the Human Fetal Lung 5'-STRETCH PLUS
λgt10 cDNA Library (Clontech) were screened with the hPDGF-C
probe according to standard procedures. Among several
positive clones, one, clone #4 was analyzed more carefully and
20   the nucleotide sequence of its insert was determined according
to standard procedures using internal and vector
oligonucleotides. The insert of clone #4 contains a partial
nucleotide sequence of the cDNA encoding the full length human
PDGF-C (hPDGF-C). The nucleotide sequence (1536 bp) of the
25   clone #4 insert is shown in Figure 3 (SEQ ID NO:4). The
translated portion of this cDNA includes nucleotides 6 to 956.
The deduced amino acid sequence of the translated portion of
the insert is illustrated in Figure 4 (SEQ ID NO:5). A
polypeptide of this deduced amino acid sequence would lack the
30   first 28 amino acid residues found in the full length hPDGF-C
polypeptide. However, this polypeptide includes a proteolytic
fragment which is sufficient to activate the PDGF alpha

receptors.    It should be noted that the first glycine (Gly) of SEQ ID NO:5 is not found in the full length hPDGF-C.

A mouse EST sequence (AI020581) was identified in a database search of the dbEST database at the NCBI in Washington, DC, which appears to encode part of a new mouse PDGF, PDGF-C.   Large parts of the mouse cDNA was obtained by PCR amplification using DNA from a mouse embryo λgt10 cDNA library as the template.   To amplify the 3' end of the cDNA, a sense primer derived from the mouse EST sequence was used (the sequence of this primer was 5'-CTT CAG TAC CTT GGA AGA G, primer 1 (SEQ ID NO:27))  To amplify the 5'end of the cDNA, an antisense primer derived from the mouse EST was used (the sequence of this primer was 5'-CGC TTG ACC AGG AGA CAA C, primer 2 (SEQ ID NO:28)). The λgt10 vector primers were sense 5'-ACG TGA ATT CAG CAA GTT CAG CCT GGT TAA (primer 3 (SEQ ID NO:29)) and antisense 5'-ACG TGG ATC CTG AGT ATT TCT TCC AGG GTA (primer 4 (SEQ ID NO:30)).  Combinations of the vector primers and the internal primers obtained from the mouse EST were used in standard PCR reactions.   The sizes of the amplified fragments were approx. 750 bp (3'-fragment) and 800 bp (5'-fragment), respectively.  These fragments were cloned into the pCR 2.1 vector and subjected to nucleotide sequences analysis using vector primers and internal primers. Since these fragments did not contain the full length sequence of mPDGF-C, a mouse liver ZAP cDNA library was screened using standard conditions.   A 261 bp $^{32}$P-labeled PCR fragment was generated for use as a probe using primers 1 and 2 and using DNA from the mouse embryo λgt10 library as the template (see above).   Several positive plaques were purified and the nucleotide sequence of the inserts were obtained following subcloning into pBluescript.  Vector specific primers and internal primers were used.   By combining the nucleotide sequence information of the generated PCR clones and the

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isolated clone, the full length amino acid sequence of mPDGF-C could be deduced (see Figure 6)(SEQ ID NO:7).

Figure 7 shows a comparative sequence alignment of the mouse and human amino acid sequences of PDGF-C (SEQ ID NOS:6

5    and 2, respectively). The alignment shows that human and mouse PDGF-Cs display an identity of about 87% with 45 amino acid replacements found among the 345 residues of the full length proteins. Almost all of the observed amino acid replacements are conservative in nature. The predicted

10   cleavage site in mPDGF-C for the signal peptidase is between residues G19 and T20. This would generate a secreted mouse peptide of 326 amino acid residues.

Figure 8 provides a schematic domain structure of mouse PDGF-C with a signal sequence (striped box), a N-terminal CUB

15   domain and the C-terminal PDGF/VEGF-homology domain (open boxes). The amino acid sequences denoted by the lines have no obvious similarities to CUB domains or to VEGF-homology domains.

20   The high sequence identity suggests that human and mouse PDGF-C have an almost identical domain structure. Amino acid sequence comparisons revealed that both mouse and human PDGF-C display a novel domain structure. Apart from the PDGF/VEGF-homology domain located in the C-terminal region in both

25   proteins (residues 164 to 345), the N-terminal region in both PDGF-Cs have a domain referred to as a CUB domain (Bork and Beckmann, J. Mol. Biol., 1993 <u>231</u>, 539-545). This domain of about 110 amino acids (amino acid residues 50-160) was originally identified in complement factors C1r/C1s, but has

30   recently been identified in several other extracellular proteins including signaling molecules such as bone morphogenic protein 1 (BMP-1) (Wozney et al.,Science, 1988 <u>242</u>, 1528-1534) as well as in several receptor molecules such

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as neuropilin-1 (NP-1) (Soker et al., Cell, 1998 _92_ 735-745). The functional roles of CUB domains are not clear but it may participate in protein-protein interactions or in interactions with carbohydrates including heparin sulfate proteoglycans.

5          Figure 9 shows the amino acid sequence alignment of the C-terminal PDGF/VEGF-homology domains of human and mouse PDGF-Cs with the C-terminal PDGF/VEGF-homology domains of PDGF/VEGF family members, $VEGF_{165}$, PlGF-2, $VEGF-B_{167}$, Pox Orf VEGF, VEGF-C, VEGF-D, PDGF-A and PDGF-B (SEQ ID NOs:8-17). Some of the
10    amino acid sequences in the N- and C-terminal regions in VEGF-C and VEGF-D have been deleted in this figure. Gaps were introduced to optimize the alignment. This alignment was generated using the method of J. Hein, (Methods Enzymol. 1990 _183_ 626-45) with PAM250 residue weight table. The boxed
15    residues indicate amino acids which match the PDGF-Cs within two distance units.

The alignment shows that PDGF-C has the expected pattern of invariant cysteine residues, a hallmark of members of this family, with one exception. Between cysteine 3 and 4,
20    normally spaced by 2 residues there is an insertion of three extra amino acids (NCA). This feature of the sequence in PDGF-C was highly unexpected.

Based on the amino acid sequence alignments in Figure 9, a phylogenetic tree was constructed and is shown in Figure 10.
25    The data show that the PDGF-C homology domain is closely related to the PDGF/VEGF-homology domains of VEGF-C and VEGF-D.

As shown in Figure 11, the amino acid sequences from several CUB-containing proteins were aligned (SEQ ID NOs:18-
30    24). The results show that the single CUB domain in human and mouse PDGF-C (SEQ ID NOs:18 and 19, respectively) displays a significant identify with the most closely related CUB domains. Sequences from human BMP-1, with 3 CUB domains (CUB1-

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3 (SEQ ID NOs:20-22)) and human neuropilin-1 with 2 CUB domains (CUB1-2)(SEQ ID NOs:23 and 24, respectively) are shown.  Gaps were introduced to optimize the alignment.  This alignment was generated using the method of J. Hein, (Methods Enzymol., 1990 <u>183</u> 626-45) with PAM250 residue weight table.

Figure 12 shows a Northern blot analysis of the expression of PDGF-C transcripts in several human tissues. The analysis shows that PDGF-C is encoded by a major transcript of approximately 3.8-3.9 kb, and a minor of 2.8 kb. The numbers to the right refer to the size of the mRNAs (in kb).  The tissue expression of PDGF-C was determined by Northern blotting using a commercial Multiple Tissue Northern blot (MTN, Clontech).  The blots were hybridized at according to the instructions from the supplier using ExpressHyb solution at 68°C for one hour (high stringency conditions), and probed with a 353 bp hPDGF-C EST probe from the fetal lung cDNA library screening as described above.  The blots were subsequently washed at 50°C in 2X SSC with 0.05% SDS for 30 minutes and at 50°C in 0.1X SSC with 0.1% SDS for an additional 40 minutes.  The blots were then put on film and exposed at -70°C.  The blots show that PDGF-C transcripts are most abundant in heart, liver, kidney, pancreas and ovary while lower levels of transcripts are present in most other tissues, including placenta, skeletal muscle and prostate. PDGF-C transcripts were below the level of detection in spleen, colon and peripheral blood leucocytes.

Figure 13 shows the regulation of PDGF-C mRNA expression by hypoxia.  Size markers (in kb) are indicated to the left in the lower panel.  The estimated sizes of PDGF-C mRNAs is indicated to the left in the upper panel (2.7 and 3.5 kbs, respectively).  To explore whether PDGF-C is induced by hypoxia, cultured human skin fibroblasts were exposed to hypoxia for 0, 4, 8 and 24 hours.  Poly(A)+ mRNA was isolated

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from cells using oligo-dT cellulose affinity purification.
Isolated mRNAs were electrophoresed through 12% agarose gels
using 4 µg of mRNA per line.  A Northern blot was made and
hybridized with a probe for PDGF-C.  The sizes of the two
5      bands were determined by hybridizing the same filter with a
mixture of hVEGF, hVEGF-B and hVEGF-C probes (Enholm et al.
Oncogene, 1997 14 2475-2483), and interpolating on the basis
of the known sizes of these mRNAs. The results shown in Figure
13 indicate that PDGF-C is not regulated by hypoxia in human
10     skin fibroblasts.

Figure 14 shows the expression of PDGF-C mRNA in human
tumor cells lines.  To explore whether PDGF-C was expressed
in human tumor cell lines, poly(A)+ mRNA was isolated from
several known tumor cell lines, the mRNAs were electrophoresed
15     through a 12% agarose gel and analyzed by Northern blotting
and hybridization with the PDGF-C probe. The results shown in
Figure 14 demonstrate that PDGF-C mRNA is expressed in several
types of human tumor cell lines such as JEG3 (a human
choriocarcinoma, ATCC #HTB-36), G401 (a Wilms tumor, ATCC
20     #CRL-1441), DAMI (a megakaryoblastic leukemia), A549 (a human
lung carcinoma, ATCC #CCL-185) and HEL (a human
erythroleukemia, ATCC #TID-180).  It is contemplated that
further growth of these PDGF-C expressing tumors can be
inhibited by inhibiting PDGF-C.  As well as using PDGF-C
25     expression as a means of identifying specific types of tumors.

Example 1:  Generation of specific antipeptide antibodies to
human PDGF-C

Two synthetic peptides were generated and then used to
30     raise antibodies against human PDGF-C.  The first synthetic
peptide corresponds to residues 29-48 of the N-terminus of
full length PDGF-C and includes an extra cysteine residue at
the N- and C-terminus: CKFQFSSNKEQNGVQDPQHERC (SEQ ID NO:31).

The second synthetic peptide corresponds to residues 230-250 of the internal region of full length PDGF-C and includes an extra cysteine residue at the C-terminus: GRKSRVVDLNLLTEEVRLYSC (SEQ ID NO:32). The two peptides were
5      each conjugated to the carrier protein keyhole limpet hemocyanin (KLH, Calbiochem) using N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) (Pharmacia Inc.) according to the instructions of the supplier. 200-300 micrograms of the conjugates in phosphate buffered saline
10     (PBS) were separately emulsified in Freunds Complete Adjuvant and injected subcutaneously at multiple sites in rabbits. The rabbits were boostered subcutaneously at biweekly intervals with the same amount of the conjugates emulsified in Freunds Incomplete Adjuvant. Blood was drawn and collected from the
15     rabbits. The sera were prepared using standard procedures known to those skilled in the art.

Example 2:    Expression of full length human PDGF-C in
mammalian cells
20         The full length cDNA encoding human PDGF-C was cloned into the mammalian expression vector, pSG5 (Stratagene, La Jolla, CA) that has the SV40 promoter. COS-1 cells were transfected with this construct and in separate transfections, with a pSG5 vector without the cDNA insert for a control,
25     using the DEAE-dextran procedure. Serum free medium was added to the transfected COS-1 cells 24 hours after the transfections and aliquots containing the secreted proteins were collected for a 24 hour period after the addition of the medium. These aliquots were subjected to precipitation using
30     ice cold 10% trichloroacetic acid for 30 minutes, and the precipitates were washed with acetone. The precipitated proteins were dissolved in SDS loading buffer under reducing conditions and separated on a SDS-PAGE gel using standard

procedures.   The separated proteins were electrotransferred onto Hybond filter and immunoblotted using a rabbit antiserum against the internal peptide of full length PDGF-C, the preparation of which is described above.   Bound antibodies were detected using enhanced chemiluminescence (ECL, Amersham Inc.).   Figure 15 shows the  results of this immunoblot.  The sample was only partially reduced and the monomer of the human PDGF-C migrated as a 55 kDa species (the lower band) and the dimer migrated as a 100 kDa species (upper band).   This indicates that the protein is secreted intact and that no major proteolytic processing occurs during secretion of the molecule in mammalian cells.

Example 3:   Expression of full length and truncated human PDGF-C in baculovirus infected Sf9 cells

The full length coding part of the human PDGF-C cDNA (970 bp) was amplified by PCR using Deep Vent DNA polymerase (Biolabs) using standard conditions and procedures.  The full length PDGF-C was amplified for 30 cycles, where each cycle consisted of one minute denaturization at 94°C, one minute annealing at 56°C and two minutes extension at 72°C.   The forward primer used was 5'CGGGATCCCGAATCCAACCTGAGTAG3' (SEQ ID NO:33).   This primer includes a BamHI site (underlined) for in  frame  cloning.   The  reverse  primer  used  was 5'GGAATTCCTAATGGTGATGGTGATGATGTTTGTCATCGTCATCTCCTCCTGTGCTC CCTCT3' (SEQ ID NO:34).   This primer includes an EcoRI site (underlined) and sequences coding for a C-terminal 6X His tag preceded by an enterokinase site.  In addition, residues 230-345 of the PDGF/VEGF homology domain (PVHD) of human PDGF-C were amplified by PCR using Deep Vent DNA polymerase (Biolabs) using standard conditions and procedures.   The residues 230-345 of the PVHD of PDGF-C were amplified for 25 cycles, where each cycle consisted of one minute denaturization at 94°C,

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four minutes annealing at 56°C and four minutes extension at 72°C. The forward primer used was 5'CGGATCCCGGAAGAAAATCCA GAGTGGTG3' (SEQ ID NO:35). This primer includes a BamHI site (underlined) for in frame cloning. The reverse primer used

5    was 5'GGAATTCCTAATGGTGATGGTGATGATGTTTGTCATCGTCATCTCCTCCTGTG CTCCCTCT-3' (SEQ ID NO:36). This primer includes an EcoRI site (underlined) and sequences coding for a C-terminal 6X His tag preceded by an enterokinase site. The PCR products were digested with BamHI and EcoRI and subsequently cloned into the

10   baculovirus expression vector, pAcGP67A. Verification of the correct sequence of the PCR products cloned into the constructs was by nucleotide sequencing. The expression vectors were then co-transfected with BaculoGold linearized baculovirus DNA into Sf9 insect cells according to the

15   manufactures protocol (Pharmingen). Recombined baculovirus were amplified several times before beginning large scale protein production and protein purification according to the manual (Pharmingen).

Sf9 cells, adapted to serum free medium, were infected

20   with recombinant baculovirus at a multiplicity of infection of about 7. Media containing the recombinant proteins were harvested 4 days after infection and were incubated with Ni-NTA-Agarose beads(Qiagen). The beads were collected in a column and after extensive washing with 50 mM sodium

25   phosphate buffer pH 8, containing 300 mM NaCl (the washing buffer), the bound proteins were eluted with increasing concentrations of imidazole (from 100 mM to 500 mM) in the washing buffer. The eluted proteins were analyzed by SDS-PAGE using 12.5% polyacrylamide gels under reducing and

30   non-reducing conditions. For immunoblotting analyses, the proteins were electrotransferred onto Hybond filters for 45 minutes.

Figures 16A-C show the isolation and partial
characterization of full length human PDGF-C protein.  In
Figure 16A, the recombinant full length protein was
visualized on the blot using antipeptide antibodies against

5        the N-terminal peptide(described above).  In Figure 16B,
the recombinant full length protein was visualized on the
blot using antipeptide antibodies against the internal
peptide (described above).  The separated proteins were
visualized by staining with Coomassie Brilliant Blue

10       (Figure 16C).  The numbers at the bottom of Figures 16A-C
refer to the concentration of imidazole used to elute the
protein from the Ni-NTA column and are expressed in
molarity (M).  Figures 16A-C also show that the full length
protein migrates as a 90 kDa species under non-reducing

15       conditions and as a 55 kDa species under reducing
conditions.  This indicates that the full length protein
was expressed as a disulfide-linked dimer.

Figures 17A-C show the analysis of the isolation and
partial characterization of a truncated form of human PDGF-

20       C containing the PDGF/VEGF homology domain only.  In Figure
17A, the immunoblot analysis of fractions eluted from the
Ni-agarose column demonstrates that the protein could be
eluted at imidazole concentrations ranging between 100-500
mM.  The eluted fractions were analyzed under non-reducing

25       conditions, and the truncated human PDGF-C was visualized
on the blot using antipeptide antibodies against the
internal peptide (described above).  Figure 17B shows the
Coomassie Brilliant Blue staining of the same fractions as
in Figure 17A.  This shows that the procedure generates

30       highly purified material migrating as a 36 kDa species.
Figure 17C shows the Coomassie Brilliant Blue staining of
non-reduced (non-red.) and reduced (red.) truncated human
PDGF-C protein.  The data show that the protein is a

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secreted dimer held together by disulfide bonds and that
the monomer migrates as a 24 kDa species.


Example 4:   Receptor binding properties of full length and
5       truncated PDGF-C

            To assess the interactions between full length and
truncated PDGF-C and the VEGF receptors, full length and
truncated PDGF-C were tested for their capacity to bind to
soluble Ig-fusion proteins containing the extracellular
10      domains of human VEGFR-1, VEGFR-2 and VEGFR-3 (Olofsson et
al., Proc. Natl. Acad. Sci. USA, 1998 95 11709-11714).  The
fusion proteins, designated VEGFR-1-Ig, VEGFR-2-Ig and
VEGFR-3-Ig, were transiently expressed in human 293 EBNA
cells.  All Ig fusion proteins were human VEGFRs.  Cells
15      were incubated for 24 hours after transfection, washed with
Dulbecco's Modified Eagle Medium (DMEM) containing 0.2%
bovine serum albumin and starved for 24 hours.  The fusion
proteins were then precipitated from the clarified
conditioned medium using protein A-Sepharose beads
20      (Pharmacia).  The beads were combined with 100 microliters
of 10X binding buffer (5% bovine serum albumin, 0.2% Tween
20 and 10 µg/ml heparin) and 900 microliter of conditioned
medium from 293 cells that had been transfected with
mammalian expression plasmids encoding full length or
25      truncated PDGF-C or control vector, then metabolically
labeled with $^{35}$S-cysteine and methionine (Promix, Amersham)
for 4 to 6 hours.  After 2.5 hours, at room temperature,
the Sepharose beads were washed 3 times with binding buffer
at 4°C, once with phosphate buffered saline and boiled in
30      SDS-PAGE buffer.  Labeled proteins that were bound to the
Ig-fusion proteins were analyzed by SDS-PAGE under reducing
conditions.  Radiolabeled proteins were detected using a
phosphorimager analyzer.  In all these analyses,

radiolabeled PDGF-C failed to show any interaction with any
of the VEGF receptors.

Next, full length and truncated PDGF-C were tested for
their capacity to bind to human PDGF receptors alpha and

5       beta by analyzing their abilities to compete with PDGF-BB
for binding to PDGF receptors. The binding experiments
were performed on porcine aortic endothelial-1 (PAE-1)
cells stably expressing the human PDGF receptors alpha and
beta (Eriksson et al., EMBO J, 1992, 11, 543-550). Binding

10      experiments were performed essentially as in Heldin et al.
(EMBO J, 1988, 7 1387-1393). Different concentrations of
human full-length and truncated PDGF-C, or human PDGF-BB
were mixed with 5 ng/ml of $^{125}I$-PDGF-BB in binding buffer
(PBS containing 1 mg/ml of bovine serum albumin). Aliquots

15      were incubated with the receptor expressing PAE-1 cells
plated in 24-well culture dishes on ice for 90 minutes.
After three washes with binding buffer, cell-bound $^{125}I$-
PDGF-BB was extracted by lysis of cells in 20 mM Tris-HCl,
pH 7.5, 10% glycerol, 1% Triton X-100. The amount of cell

20      bound radioactivity was determined in a gamma-counter. A
standard curve for the binding of $^{125}I$-labeled PDGF BB
homodimers to PAE-1 cells expressing PDGF alpha-receptor is
shown in Figure 18. An increasing excess of the unlabeled
protein added to the incubations competed efficiently with

25      cell association of the radiolabeled tracer.

Figure 19 graphically shows that the truncated PDGF-C
efficiently competed for binding to the PDGF alpha-
receptor, while the full length protein did not. Both the
full length and truncated proteins failed to compete for

30      binding to the PDGF beta-receptor.


Example 5:   PDGF alpha-receptor Phosphorylation

To test if PDGF-C causes increased phosphorylation of

the PDGF alpha-receptor, full length and truncated PDGF-C
were tested for their capacity to bind to the PDGF alpha-
receptor and stimulate increased phosphorylation.  Serum-
starved porcine aortic endothelial (PAE) cells stably

5    expressing the human PDGF alpha-receptor were incubated on
ice for 90 minutes with PBS supplemented with 1 mg/ml BSA
and 10ng/ml of PDGF-AA, 100ng/ml of full length human PDGF-
CC homodimers (flPDGF-CC), 100ng/ml of truncated PDGF-CC
homodimers (cPDGF-CC), or a mixture of 10ng/ml of PDGF-AA

10   and 100ng/ml of truncated PDGF-CC.  Full length and
truncated PDGF-CC homodimers were produced as described
above.  Sixty minutes after the addition of the
polypeptides, the cells were lysed in lysis buffer (20 mM
tris-HCl, pH 7.5, 0.5% Triton X-100, 0.5% deoxycholic acid,

15   10 mM EDTA, 1 mM orthovanadate, 1 mM PMSF 1% Trasylol).
The PDGF alpha-receptors were immunoprecipitated from
cleared lysates with rabbit antisera against the human PDGF
alpha-receptor (Eriksson et al., EMBO J, 1992 11 543-550).
The precipitated receptors were applied to a SDS-PAGE gel.

20   After SDS gel electrophoresis, the precipitated receptors
were transferred to nitrocellulose filters, and the filters
were probed with anti-phosphotyrosine antibody PY-20,
(Transduction Laboratories).  The filters were then
incubated with horseradish peroxidase-conjugated anti-mouse

25   antibodies.  Bound antibodies were detected using enhanced
chemiluminescence (ECL, Amersham Inc).  The filters were
then stripped and reprobed with the PDGF alpha-receptor
rabbit antisera, and the amount of receptors was determined
by incubation with horseradish peroxidase-conjugated anti-

30   rabbit antibodies.  Bound antibodies were detected using
enhanced chemiluminescence (ECL, Amersham Inc).  The
probing of the filters with PDGF alpha-receptor antibodies
confirmed that equal amounts of the receptor were present

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in all lanes.  PDGF-AA is included in the experiment as a
control.  Figure 20 shows that truncated, but not full
length PDGF-CC, efficiently induced PDGF alpha-receptor
tyrosine phosphorylation.  This indicates that truncated
5      PDGF-CC is a potent PDGF alpha-receptor agonist.


Example 6:   Mitogenicity of PDGF-C for Fibroblasts
        Figure 21 shows the mitogenic activities of truncated
and full length PDGF-CC on fibroblasts.  The assay was
10     performed essentially as described in Mori et al., J. Biol.
Chem., 1991 266 21158-21164.  Serum starved human foreskin
fibroblasts were incubated for 24 hours with 1 ml of serum-
free medium supplemented with 1 mg/ml BSA and 3ng/ml,
10ng/ml or 30ng/ml of full length PDGF-CC (flPDGF-CC),
15     truncated PDGF-CC (cPDGF-CC) or PDGF-AA in the presence of
0.2 μmCi [3H]thymidine.  After trichloroacetic acid (TCA)
precipitation, the incorporation of [3H]thymidine into DNA
was determined using a beta-counter.  The results show that
truncated PDGF-CC, but not full length PDGF-CC, is a potent
20     mitogen for fibroblasts.  PDGF-AA is included in the
experiment as a control.


        PDGF-C does not bind to any of the known VEGF
receptors.  PDGF-C is the only VEGF family member, thus
25     far, which can bind to and increase phosphorylation of the
PDGF alpha-receptor.  PDGF-C is also the only VEGF family
member, thus far, to be a potent mitogen of fibroblasts.
These characteristics indicate that the truncated form of
PDGF-C may not be a VEGF family member, but instead a novel
30     PDGF.  Furthermore, the full length protein is likely to be
a latent growth factor that needs to be activated by
proteolytic processing to release the active PDGF/VEGF
homology domain.  A putative proteolytic site is the

dibasic motif found in residues 231-234 in the full length
protein, residues -R-K-S-R-.  This site is structurally
conserved in a comparison between mouse and human PDGF-Cs
(Figure 7).  Preferred proteases include, but are not

5    limited to, Factor X and enterokinase.  The N-terminal CUB
domain may be expressed as an inhibitory domain which might
be used to localize this latent growth factor in some
extracellular compartment (for example the extracellular
matrix) and which is removed by limited proteolysis when

10   need, for example during embryonic development, tissue
regeneration, tissue remodelling including bone
remodelling, active angiogenesis, tumor progression, tumor
invasion, metastasis formation and/or wound healing.


15   Example 7:  PDGF Receptors Binding of Truncated PDGF-C
          To assess the interactions between truncated PDGF-C
and the PDGF alpha and beta receptors, truncated PDGF-C was
tested for its capacity to bind to porcine aortic
endothelial-1 (PAE-1) cells expressing PDGF alpha or beta

20   receptors, respectively (Eriksson et al., EMBO J, 1992, 11
543-550).  The binding experiments were performed
essentially as described in Heldin et al. (EMBO J, 1988, 7
1387-1393).  Five micrograms of truncated PDGF-C protein in
ten microliters of sodium borate buffer was radiolabeled

25   using the Bolton-Hunter reagent (Amersham) to a specific
activity of 4 x $10^5$ cpm/ng.  Different concentrations of
radiolabeled truncated PDGF-C, with or without added
unlabeled protein, in binding buffer (PBS containing
1 mg/ml of bovine serum albumin) was added to the receptor

30   expressing PAE-1 cells plated in 24-well culture dishes on
ice for 90 minutes.  After three washes with binding
buffer, cell-bound $^{125}$I-labeled PDGF-C was extracted by
lysis of cells in 20 mM Tris-HCl, pH 7.5, 10% glycerol, 1%

Triton X-100.  The amount of cell-bound radioactivity was
determined in a gamma-counter.  Non-specific binding was
estimated by including a 100-fold molar excess of truncated
PDGF-C in some experiments.  All binding data represents
5      the mean of triplicate analyses and the experimental
variation in the experiment varied between 10-15%.  As seen
in Figure 22, truncated PDGF-C binds to cells expressing
PDGF alpha receptors, but not to beta receptor expressing
cells.  The binding was specific as radiolabeled PDGF-C was
10     quantitatively displaced by a 100-fold molar excess of
unlabeled protein.

Example 8: Protease Effects on Full length PDGF-C
        To demonstrate that full length PDGF-C can be
15     activated by limited proteolysis to release the PDGF/VEGF
homology domain from the CUB domain, the full length
protein was digested with different proteases.  For
example, full length PDGF-C was digested with plasmin in 20
mM Tris-HCl (pH 7.5) containing 1 mM $CaCl_2$, 1 mM $MgCl_2$ and
20     0.01% Tween 20 for 1.5 to 4.5 hours at 37°C using two to
three units of plasmin (Sigma) per ml.  The released domain
essentially corresponded in size to the truncated PDGF-C
species previously produced in insect cells.
Plasmin-digested PDGF-C and undigested full length PDGF-C
25     were applied to a SDS-PAGE gel under reducing conditions.
After SDS-PAGE gel electrophoresis, the respective proteins
were transferred to a nitrocellulose filter, and the filter
was probed using a rabbit antipeptide antiserum to residues
230-250 in full length protein (residues GRKSRVVDLNLLTEEVR
30     LYSC (SEQ ID NO:37) located in just N-terminal to the
PDGF/VEGF homology domain).  Bound antibodies were detected
using enhanced chemiluminescence (ECL, Amersham Inc).
Figure 23 shows the immunoblot with a 55 kDa undigested

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full length protein and the plasmin-generated 26-28 kDa
species.


5       Example 9: PDGF alpha Receptors Binding of Plasmin-digested
        PDGF-C
                To assess the interactions between plasmin-digested
        PDGF-C and the PDGF alpha receptors, plasmin-digested PDGF-
        C was tested for its capacity to bind to porcine aortic
10      endothelial-1 (PAE-1) cells expressing PDGF alpha receptors
        (Eriksson et al., EMBO J, 1992, 11 543-550). The receptor
        binding analyses were performed essentially as in Example 7
        using 30 ng/ml of $^{125}$I-labeled truncated PDGF-C as the
        tracer. As seen in Figure 24, increasing concentrations of
15      plasmin-digested PDGF-C efficiently competed for binding to
        the PDGF alpha receptors. In contrast, undigested full
        length PDGF-C failed to compete for receptor binding.
        These data indicate that full length PDGF-C is a latent
        growth factor unable to interact with PDGF alpha receptors
20      and that limited proteolysis, which releases the C-terminal
        PDGF/VEGF homology domain, is necessary to generate an
        active PDGF alpha receptor ligand/agonist.


        Example 10:  Cloning and expression of the Human PDGF-C CUB
25      domain
                A human PDGF-C 430 bp cDNA fragment encoding the CUB
        domain (amino acid residues 23 - 159 in full length PDGF-C)
        was amplified by PCR using Deep Vent DNA polymerase
        (Biolabs) using standard conditions and procedures. The
30      forward primer used was 5´cgggatcccgaatccaacctgagtag3´ (SEQ
        ID NO:38). This primer includes a BamHI site (underlined)
        for in clone frame cloning. The reverse primer used was
        5´ccggaattcctaatggtgatggtgatgatgtttgtcatcgtcgtcgacaatgttgta

gtg3′ (SEQ ID NO:39). This primer includes an EcoRI site
(underlined) and sequences coding for a C-terminal 6x His
tag preceded by an enterokinase site. The amplified PCR
fragment was subsequently cloned into a pACgp67A transfer

5      vector. Verification of the correct sequence of the
expression construct, CUB-pACgp67A, was by automatic
nucleotide sequencing. The expression vectors were then
co-transfected with BaculoGold linearized baculovirus DNA
into Sf9 insect cells according to the manufacture's

10     protocol (Pharmingen). Recombined baculovirus were
amplified several times before beginning large scale
protein production and protein purification according to
the manual (Pharmingen).

        Sf9 cells, adapted to serum free medium, were infected

15     with recombinant baculovirus at a multiplicity of infection
of about 7. Media containing the recombinant proteins were
harvested 72 hours after infection and were incubated with
Ni-NTA-Agarose beads(Qiagen) overnight at 4°C. The beads
were collected in a column and after extensive washing with

20     50 mM sodium phosphate buffer pH 8, containing 300 mM NaCl
(the washing buffer), the bound proteins were eluted with
increasing concentrations of imidazole (from 100 mM to 400
mM) in the washing buffer. The eluted proteins were
analyzed by SDS-PAGE using a polyacrylamide gel under

25     reducing and non-reducing conditions.

        Figure 25 shows the results from Coomassie blue
staining of the gel. The human PDGF-C CUB domain is a
disulfide-linked homodimer with a molecular weight of about
55 KD under non-reducing conditions, while two monomers of

30     about 25 and 30 KD respectively are present under reducing
conditions. The heterogeneity is probably due to
heterogenous glycosylation of the two putative N-linked
glycosylation sites present in the CUB domain at amino acid

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positions 25 and 55.  A protein marker lane is shown to the
left in the figure.


5      Example 11: Localization of PDGF-C transcripts in
       developing mouse embryos
               To gain insight into the biological function of PDGF-
       C, PDGF-C expression in mouse embryos was localized by non-
       radioactive in situ hybridization in tissue sections from
10     the head (Figures 26A-26S) and urogenital tract (Figures
       26T-26V) regions.  The non-radioactive in situ
       hybridization employed protocols and PDGF-A and PDGFR-alpha
       probes are described in Boström et al., Cell, 1996 85 863-
       873, which is hereby incorporated by reference.  The PDGF-C
15     probe was derived from a mouse PDGF-C cDNA.  The
       hybridization patterns shown in Figures 26A-26V are for
       embryos aged E16.5, but analogous patterns are seen at
       E14.5, E15.5 and E17.5.  Sense probes were used as controls
       and gave no consistent pattern of hybridization to the
20     sections.
               Figure 26A shows the frontal section through the mouth
       cavity at the level of the tooth anlagen (t).  The arrows
       point to sites of PDGF-C expression in the oral ectoderm.
       Also shown is the tongue (to).  Figures 26B-26D show PDGF-C
25     expression in epithelial cells of the developing tooth
       canal.  Individual cells are strongly labeled in this area
       (arrow in Figure 26D), as well as in the developing palate
       ectoderm (right arrow in Figure 26C).  Figure 26E shows the
       frontal section through the eye, where PDGF-C expression is
30     seen in the hair follicles (double arrow) and in the
       developing eyelid.  Also shown is the retina (r).  In
       Figures 26F and 26G, the PDGF-C expression is found in the
       outer root sheath of the developing hair follicle

epithelium. In Figure 26H, PDGF-C expression is shown in
the developing eyelid. There is an occurrence of
individual strongly PDGF-C positive cells in the developing
opening. Also shown is the lens (l). In Figure 26I, PDGF-
5       C expression in the developing lacrimal gland is shown by
the arrow. In Figure 26J, PDGF-C expression in the
developing external ear is shown. Expression is seen in
the external auditory meatus (left arrow) and in the
epidermal cleft separating the prospective auricle (e).
10      Figures 26K and 26L show PDGF-C expression in the cochlea.
Expression is seen in the semi-circular canals (arrows in
26K). There is a polarized distribution of PDGF-C mRNA in
epithelial cells adjacent to the developing hair cells
(arrow in 26L). Figures 26M and 26N show PDGF-C expression
15      in the oral cavity. Horizontal sections show expression in
buccal epithelium (arrows in 26M) and in the forming cleft
between the lower lip buccal and the gingival epithelium
(arrows in 26N). Also shown is the tooth anlagen (t) and
the tongue (to). Figures 26O and 26P show PDGF-C
20      expression in the developing nostrils, shown on horizontal
sections. PDGF-C expression appears strongest before
stratification of the epithelium and the formation of the
canal proper (arrows in 26O and 26P). Also shown is the
developing nostrils (n). Figures 26Q-26S show PDGF-C
25      expression in developing salivary glands and ducts. Figure
26Q is the sublingual gland. Figures 26R and 26S show the
maxillary glands, the salivary gland (sg) and the salivary
duct (sd). Figures 26T-26V show the expression of PDGF-C
in the urogenital tract. Figure 26T shows the expression
30      of PDGF-C in the developing kidney metanephric mesoderm.
Figure 26U shows the expression of PDGF-C in the urethra
(ua) and in epithelium surrounding the developing penis.

Figure 26V shows the PDGF-C expression in the developing
ureter (u).

Example 12: PDGF-C, PDGF-A and PDGFR-alpha Expression in
the Developing Kidney
        One of the strongest sites of PDGF-C expression is the
developing kidney and so expression of PDGF-C, PDGF-A and
PDGFR-alpha was looked at in the developing kidney.
Figures 27A-27F show the results of non-radioactive *in situ*
hybridization demonstrating the expression (blue staining
in unstained background visualized using DIC optics) of
mRNA for PDGF-C (Figures 27A and 27B), PDGF-A (Figures 27C
and 27D) and PDGFR-alpha (Figures 27E and 27F) in E16.5
kidneys.  The white hatched line in Figures 27B, 27D and
27F outlines the cortex border.  The bar in Figures 27A,
27C and 27E represents 250 μm, and in Figures 27B, 27D and
27F represents 50 μm.
        PDGF-C expression is seen in the metanephric
mesenchyme (mm in Figure 27A), and appears to be
upregulated in the condensed mesenchyme (arrows in Figure
27B) undergoing epithelial conversion as a prelude to
tubular development, which is situated on each side of the
ureter bud (ub).  PDGF-C expression remains at lower levels
in the early nephronal epithelial aggregates (arrowheads in
B), but is absent from mature glomeruli (gl) and tubular
structures.
        PDGF-A expression is not seen in these early
aggregates, but is strong in later stages of tubular
development (Figures 24C and 24D).  PDGF-A is expressed in
early nephronal epithelial aggregates (arrowheads in Figure
27D), but once the nephron is developed further, PDGF-A
expression becomes restricted to the developing Henle's
loop (arrow in Figure 27D).  The strongest expression is

seen in the Henle's loops in the developing marrow (arrows
in Figure 27C). The branching ureter (u) and the ureter
bud (ub) is negative for PDGF-A.

    Thus, the PDGF-C and PDGF-A expression patterns in the
5       developing nephron are spatially and temporally distinct.
PDGF-C is expressed in the earliest stages (mesenchymal
aggregates) and PDGF-A in the latest stages (Henle's loop
formation) of nephron development.

    PDGFR-alpha is expressed throughout the mesenchyme of
10    the developing kidney (Figures 27E and 27F) and may hence
be targeted by both PDGF-C and PDGF-A. PDGF-B expression
is also seen in the developing kidney, but occurs only in
vascular endothelial cells. PDGFR-beta expression takes
place in perivascular mesenchyme, and its activation by
15    PDGF-B is critical for mesangial cell recruitment into
glomeruli.

    These results demonstrate that PDGF-C expression
occurs in close spatial relationship to sites of PDGFR-
alpha expression, and are distinct from the expression
20    sites of PDGF-A or PDGF-B. This indicates that PDGF-C may
act through PDGFR-alpha *in vivo*, and may have functions
that are not shared with PDGF-A and PDGF-B.

    Since the unique expression pattern of PDGF-C in the
developing kidney indicates a function as a PDGFR-alpha
25    agonist separate from that of PDGF-A or -B, a comparison
was made to the histology of embryonic day 16.5 kidneys
from PDGFR-alpha knockout mice (Figures 28B and 28F) with
kidneys from wildtype (Figures 28A and 28C), PDGF-A
knockout (Figure 28D) and PDGF-A/PDGF-B double knockout
30    (Figure 28E) mice. The bar in Figures 28A and 28B
represents 250 mm, and in Figures 28C-28F represents 50 µm.

    Heterozygote mutants of PDGF-A, PDGF-B and PDGFR-alpha
(Boström *et al.*, Cell, 1996 <u>85</u> 863-873; Levéen *et al.*,

Genes Dev., 1994 8 1875-1887; Soriano et al., Development,
1997 124 2691-70) were bred as C57Bl6/129sv hybrids and
intercrossed to produce homozygous mutant embryos.  PDGF-
A/PDGF-B heterozygote mutants were crossed to generate

5       double PDGF-A/PDGF-B knockout embryos.  Due to a high
degree of lethality of PDGF-A -/- embryos before E10
(Boström et al., Cell, 1996 85 863-873), the proportion of
double knockout E16.5 embryos obtained in such crosses were
less than 1/40.  The histology of kidney phenotypes was

10      verified on at least two embryos of each genotype, except
the PDGF-A/PDGF-B double knockout for which only a single
embryo was obtained.

        It is interesting that there is lack of interstitial
mesenchyme in the cortex of PDGFR-alpha  -/- kidney (arrows

15      in Figure 28A and asterisk in Figure 28F) and the presence
of interstitial mesenchyme in all other genotypes
(asterisks in Figure 28C-E).  The branching ureter (u) and
the metanephric mesenchyme (mm) and its epithelial
derivatives appear normal in all mutants.  The abnormal

20      glomerulus in the PDGF-A/PDGF-B double knockout reflect
failure of mesangial cell recruitment into the glomerular
tuft due to the absence of PDGF-B.

        These results indicate that PDGFR-alpha knockouts have
a kidney phenotype, which is not seen in PDGF-A or PDGF-

25      A/PDGF-B knockouts, hence potentially reflecting loss of
signaling by PDGF-C.  The phenotype consists of the marked
loss of interstitial mesenchyme in the developing kidney
cortex.  The cells lost in PDGFR-alpha -/- kidneys are thus
normally PDGFR-alpha positive cells adjacent to the site of

30      expression of PDGF-C.


BIOASSAYS TO DETERMINE THE FUNCTION OF PDGF-C
        Assays are conducted to evaluate whether PDGF-C has

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similar activities to PDGF-A, PDGF-B, VEGF, VEGF-B, VEGF-C
and/or VEGF-D in relation to growth and/or motility of
connective tissue cells, fibroblasts, myofibroblasts and
glial cells; to endothelial cell function; to angiogenesis;
5      and to wound healing.  Further assays may also be
performed, depending on the results of receptor binding
distribution studies.


10     **I.    Mitogenicity of PDGF-C for Endothelial Cells**
       To test the mitogenic capacity of PDGF-C for
endothelial cells, the PDGF-C polypeptide is introduced
into cell culture medium containing 5% serum and applied to
bovine aortic endothelial cells (BAEs) propagated in medium
15     containing 10% serum.  The BAEs are previously seeded in
24-well dishes at a density of 10,000 cells per well the
day before addition of the PDGF-C.  Three days after
addition of this polypeptide the cells were dissociated
with trypsin and counted.  Purified VEGF is included in the
20     experiment as positive control.


       **II.   Assays of Endothelial Cell Function**
       a)     *Endothelial cell proliferation*
       Endothelial cell growth assays are performed by
25     methods well known in the art, *e.g.* those of Ferrara &
Henzel, Nature, 1989 <u>380</u> 439-443, Gospodarowicz *et al.*,
Proc. Natl. Acad. Sci. USA; 1989 <u>86</u> 7311-7315, and/or
Claffey *et al.*, Biochem. Biophys. Acta, 1995 <u>1246</u> 1-9.


30     b)     *Cell adhesion assay*
       The effect of PDGF-C on adhesion of polymorphonuclear
granulocytes to endothelial cells is tested.

c)    *Chemotaxis*

The standard Boyden chamber chemotaxis assay is used to test the effect of PDGF-C on chemotaxis.

5    d)    *Plasminogen activator assay*

Endothelial cells are tested for the effect of PDGF-C on plasminogen activator and plasminogen activator inhibitor production, using the method of Pepper *et al.*, Biochem. Biophys. Res. Commun., 1991 <u>181</u> 902-906.

10

e)    *Endothelial cell Migration assay*

The ability of PDGF-C to stimulate endothelial cells to migrate and form tubes is assayed as described in Montesano *et al.*, Proc. Natl. Acad. Sci. USA, 1986 <u>83</u> 7297-

15    7301.  Alternatively, the three-dimensional collagen gel assay described in Joukov *et al.*, EMBO J., 1996 <u>15</u> 290-298 or a gelatinized membrane in a modified Boyden chamber (Glaser *et al.*, Nature, 1980 <u>288</u> 483-484) may be used.

20    **III. Angiogenesis Assay**

The ability of PDGF-C to induce an angiogenic response in chick chorioallantoic membrane is tested as described in Leung *et al.*, Science, 1989 <u>246</u> 1306-1309.  Alternatively the rat cornea assay of Rastinejad *et al.*, Cell, 1989 <u>56</u>

25    345-355 may be used; this is an accepted method for assay of *in vivo* angiogenesis, and the results are readily transferrable to other *in vivo* systems.

30    **IV.  Wound Healing**

The ability of PDGF-C to stimulate wound healing is tested in the most clinically relevant model available, as

described in Schilling *et al.*, Surgery, 1959 <u>46</u> 702-710 and
utilized by Hunt *et al.*, Surgery, 1967 <u>114</u> 302-307.

**V.    The Haemopoietic System**

5          A variety of *in vitro* and *in vivo* assays using
specific cell populations of the haemopoietic system are
known in the art, and are outlined below.  In particular a
variety of *in vitro* murine stem cell assays using
fluorescence-activated cell sorter to purified cells are

10      particularly convenient:

a)    *Repopulating Stem Cells*
          These are cells capable of repopulating the bone
marrow of lethally irradiated mice, and have the Lin⁻, $Rh^{hl}$,

15      Ly-6A/E⁺, c-kit⁺ phenotype.  PDGF-C is tested on these cells
either alone, or by co-incubation with other factors,
followed by measurement of cellular proliferation by $^{3}$H-
thymidine incorporation.

20      b)    *Late Stage Stem Cells*
          These are cells that have comparatively little bone
marrow repopulating ability, but can generate D13 CFU-S.
These cells have the Lin⁻, $Rh^{hl}$, Ly-6A/E⁺, c-kit⁺ phenotype.
PDGF-C is incubated with these cells for a period of time,

25      injected into lethally irradiated recipients, and the
number of D13 spleen colonies enumerated.

c)    *Progenitor-Enriched Cells*
          These are cells that respond *in vitro* to single growth

30      factors and have the Lin⁻, $Rh^{hl}$, Ly-6A/E⁺, c-kit⁺ phenotype.
This assay will show if PDGF-C can act directly on
haemopoietic progenitor cells.  PDGF-C is incubated with

these cells in agar cultures, and the number of colonies present after 7-14 days is counted.

## VI.  Atherosclerosis

Smooth muscle cells play a crucial role in the development or initiation of atherosclerosis, requiring a change of their phenotype from a contractile to a synthetic state.  Macrophages, endothelial cells, T lymphocytes and platelets all play a role in the development of atherosclerotic plaques by influencing the growth and phenotypic modulations of smooth muscle cell.  An *in vitro* assay using a modified Rose chamber in which different cell types are seeded on to opposite cover slips measures the proliferative rate and phenotypic modulations of smooth muscle cells in a multicellular environment, and is used to assess the effect of PDGF-C on smooth muscle cells.

## VII. Metastasis

The ability of PDGF-C to inhibit metastasis is assayed using the Lewis lung carcinoma model, for example using the method of Cao *et al.*, J. Exp. Med., 1995 182 2069-2077.

## VIII. Migration of Smooth Muscle Cells

The effects of the PDGF-C on the migration of smooth muscle cells and other cells types can be assayed using the method of Koyama *et al.*, J. Biol. Chem., 1992 267 22806-22812.

## IX.  Chemotaxis

The effects of the PDGF-C on chemotaxis of fibroblast, monocytes, granulocytes and other cells can be assayed

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using the method of Siegbahn et al., J. Clin. Invest., 1990
85 916-920.

## X.    PDGF-C in Other Cell Types

5          The effects of PDGF-C on proliferation,
differentiation and function of other cell types, such as
liver cells, cardiac muscle and other cells, endocrine
cells and osteoblasts can readily be assayed by methods
known in the art, such as $^3$H-thymidine uptake by in vitro
10    cultures.  Expression of PDGF-C in these and other tissues
can be measured by techniques such as Northern blotting and
hybridization or by in situ hybridization.

## XI.    Construction of PDGF-C Variants and Analogues

15         PDGF-C is a member of the PDGF family of growth
factors which exhibits a high degree of homology to the
other members of the PDGF family.  PDGF-C contains eight
conserved cysteine residues which are characteristic of
20    this family of growth factors.  These conserved cysteine
residues form intra-chain disulfide bonds which produce the
cysteine knot structure, and inter-chain disulfide bonds
that form the protein dimers which are characteristic of
members of the PDGF family of growth factors.  PDGF-C
25    interacts with a protein tyrosine kinase growth factor
receptor.
           In contrast to proteins where little or nothing is
known about the protein structure and active sites needed
for receptor binding and consequent activity, the design of
30    active mutants of PDGF-C is greatly facilitated by the fact
that a great deal is known about the active sites and
important amino acids of the members of the PDGF family of
growth factors.

Published articles elucidating the structure/activity
relationships of members of the PDGF family of growth
factors include for PDGF: Oestman *et al.*, J. Biol. Chem.,
1991 _266_ 10073-10077; Andersson *et al.*, J. Biol. Chem.,

5      1992 _267_ 11260-1266; Oefner *et al.*, EMBO J., 1992 _11_ 3921-
3926; Flemming *et al.*, Molecular and Cell Biol., 1993 _13_
4066-4076 and Andersson *et al.*, Growth Factors, 1995 _12_
159-164; and for VEGF: Kim *et al.*, Growth Factors, 1992 _7_
53-64; Pötgens *et al.*, J. Biol. Chem., 1994 _269_ 32879-32885

10     and Claffey *et al.*, Biochem. Biophys. Acta, 1995 _1246_ 1-9.
From these publications it is apparent that because of the
eight conserved cysteine residues, the members of the PDGF
family of growth factors exhibit a characteristic knotted
folding structure and dimerization, which result in

15     formation of three exposed loop regions at each end of the
dimerized molecule, at which the active receptor binding
sites can be expected to be located.

Based on this information, a person skilled in the
biotechnology arts can design PDGF-C mutants with a very

20     high probability of retaining PDGF-C activity by conserving
the eight cysteine residues responsible for the knotted
folding arrangement and for dimerization, and also by
conserving, or making only conservative amino acid
substitutions in the likely receptor sequences in the

25     loop 1, loop 2 and loop 3 region of the protein structure.
The formation of desired mutations at specifically
targeted sites in a protein structure is considered to be a
standard technique in the arsenal of the protein chemist
(Kunkel *et al.*, Methods in Enzymol., 1987 _154_ 367-382).

30     Examples of such site-directed mutagenesis with VEGF can be
found in Pötgens *et al.*, J. Biol. Chem., 1994 _269_ 32879-
32885 and Claffey *et al.*, Biochem. Biophys. Acta, 1995 _1246_

1-9.   Indeed, site-directed mutagenesis is so common that kits are commercially available to facilitate such procedures (e.g. Promega 1994-1995 Catalog., Pages 142-145).

5          The connective tissue cell, fibroblast, myofibroblast and glial cell growth and/or motility activity, the endothelial cell proliferation activity, the angiogenesis activity and/or the wound healing activity of PDGF-C mutants can be readily confirmed by well established

10      screening procedures.  For example, a procedure analogous to the endothelial cell mitotic assay described by Claffey et al., (Biochem. Biophys. Acta., 1995 1246 1-9) can be used.  Similarly the effects of PDGF-C on proliferation of other cell types, on cellular differentiation and on human

15      metastasis can be tested using methods which are well known in the art.

        The foregoing description and examples have been set forth merely to illustrate the invention and are not intended to be limiting.  Since modifications of the

20      disclosed embodiments incorporating the spirit and substance of the invention may occur to persons skilled in the art, the invention should be construed broadly to include all variations falling within the scope of the appended claims and equivalents thereof.

What is claimed is:

1.    An isolated nucleic acid molecule comprising a polynucleotide sequence having at least 85% identity with the sequence of Figure 1, 3 or 5 (SEQ ID NOs: 2, 4 and 6, respectively).

2.    An isolated nucleic acid molecule according to claim 1, wherein the sequence identity is at least 90%.

3.    An isolated nucleic acid molecule according to claim 1, wherein the sequence identity is at least 95%.

4.    An isolated nucleic acid molecule according to claim 1, wherein said nucleic acid is a cDNA.

5.    An isolated nucleic acid molecule according to claim 1, wherein said nucleic acid is a mammalian polynucleotide.

6.    An isolated nucleic acid molecule according to claim 5, wherein said nucleic acid is a murine polynucleotide.

7.    An isolated nucleic acid molecule according to claim 6, comprising the sequence shown in Figure 5 (SEQ ID NO:6).

8.    An isolated nucleic acid molecule according to claim 5, wherein said nucleic acid is a human polynucleotide.

9.   An isolated nucleic acid molecule according to
claim 8, comprising the sequence shown in Figure 1 or
Figure 3 (SEQ ID NOs: 2 and 4, respectively).

10.   An isolated nucleic acid molecule which encodes a
polypeptide molecule comprising an amino acid sequence
having at least 85% identity with the amino acid sequence
of Figure 2 (SEQ ID NO:3) or the amino acid sequence of
Figure 4 (SEQ ID NO:5), or a fragment or analog thereof
having the biological activity of PDGF-C.

11.   An isolated nucleic acid molecule according to
claim 10, wherein the amino acid sequence identity is at
least 90%.

12.   An isolated nucleic acid molecule according to
claim 10, wherein the amino acid sequence identity is at
least 95%.

13.   An isolated nucleic acid molecule which codes for
a polypeptide which comprises the amino acid sequence
              PXCXXVXRCGGXXXCC   (SEQ ID NO:1).

14.   A vector comprising a nucleic acid according to
claim 1, which nucleic acid is operably linked with a
promoter sequence.

15.   A vector according to claim 14, wherein said
vector is a eukaryotic vector.

16.   A vector according to claim 14, wherein said
vector is a prokaryotic vector.

17.    A vector according to claim 14, wherein said
vector is a plasmid.

18.    A vector according to claim 14, wherein said
vector is a baculovirus vector.

19.    A method of making a vector which expresses a
polypeptide comprising an amino acid sequence having at
least 85% identity with the amino acid sequence of Figure 2
(SEQ ID NO:3) or the amino acid sequence of Figure 6 (SEQ
ID NO:7), or fragment or analog thereof having the
biological activity of PDGF-C, said method comprising
incorporating an isolated nucleic acid according to claim
1, claim 10 or claim 13, into said vector in operatively
linked relation with a promoter.

20.    A host cell transformed or transfected with a
vector according to claim 14.

21.    A host cell according to claim 20, wherein said
host cell is a eukaryotic cell.

22.    A host cell according to claim 20, wherein said
host cell is a COS cell.

23.    A host cell according to claim 20, wherein said
host cell is a prokaryotic cell.

24.    A host cell according to claim 20, wherein said
host cell is a 293EBNA cell.

25.    A host cell according to claim 20, wherein said
host cell is an insect cell.

26.  A host cell transformed or transfected with a
vector comprising a nucleic acid sequence according to
claim 1, operatively linked to a promoter, such that said
host cell expresses a polypeptide comprising an amino acid
sequence having at least 85% identity with the amino acid
sequence of Figure 2 (SEQ ID NO:3) or the amino acid
sequence of Figure 6 (SEQ ID NO:7), or a fragment or analog
thereof having the biological activity of PDGF-C.

27.  An isolated polypeptide comprising at least 85%
identity with the amino acid sequence of Figure 2 (SEQ ID
NO:3) or the amino acid sequence of Figure 6 (SEQ ID NO:7),
or a fragment or analog thereof having the biological
activity of PDGF-C.

28.  An isolated polypeptide according to claim 27,
wherein said polypeptide is a murine polypeptide.

29.  An isolated polypeptide according to claim 27,
wherein said polypeptide is a human polypeptide.

30.  An isolated polypeptide according to claim 27,
wherein said polypeptide has the ability to stimulate
and/or enhance proliferation and/or differentiation and/or
growth and/or motility of cells expressing a PDGF-C
receptor.

31.  An isolated polypeptide produced by expression of
a polynucleotide comprising the polynucleotide sequence
having at least 85% identity with the Figure 1, 3 or 5 (SEQ
ID NOs: 2, 4 or 6, respectively), or a polynucleotide which
hybridizes under stringent conditions with at least one of
the foregoing DNA sequences.

32.  An isolated polypeptide which comprises the
characteristic sequence
            PXCXXVXRCGGXXXRCC    (SEQ ID NO:1).


33.  An isolated polypeptide dimer comprising a
polypeptide according to claim 27.


34.  An isolated polypeptide dimer according to claim
33, wherein said polypeptide dimer is a homodimer of said
polypeptide.


35.  An isolated polypeptide dimer according to claim
33, wherein said polypeptide dimer is a heterodimer of said
polypeptide and VEGF, VEGF-B, VEGF-C, VEGF-D, PDGF-A, PDGF-
B or PlGF.


36.  An isolated polypeptide dimer according to claim
33, wherein said polypeptide dimer is a disulfide-linked
dimer.


37.  A pharmaceutical composition comprising an
effective cell proliferation promoting amount of a
polypeptide according to claim 27, claim 31 or claim 32,
and at least one further growth factor selected from the
group consisting of VEGF, VEGF-B, VEGF-C, VEGF-D, PDGF-A,
PDGF-B or PlGF.


38.  A pharmaceutical composition according to claim
37, further comprising heparin.


39.  A pharmaceutical composition comprising an
effective cell proliferation promoting amount of an
isolated polypeptide according to claim 27, claim 31 or

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claim 32, and at least one pharmaceutical carrier or
diluent.

40.   A pharmaceutical composition comprising a PDGF
receptor stimulating amount of an isolated polypeptide
according to claim 27, claim 31 or claim 32, and at least
one pharmaceutical carrier or diluent.

41.   A pharmaceutical composition comprising an
effective connective tissue or wound healing stimulating
amount of an isolated polypeptide according to claim 27,
claim 31 or claim 32, and at least one pharmaceutical
carrier or diluent.

42.   A means for amplifying a polynucleotide according
to claim 1 in a test sample, said means comprising at least
one pair of primers complementary to a nucleic acid
according to claim 1.

43.   A means for amplifying a polynucleotide as
according to claim 1 in a test sample, said means
comprising a polymerase and at least one pair of primers
complementary to a nucleic acid according to claim 1, for
amplifying the polynucleotide by polymerase chain reaction
in order to facilitate a sequence comparison of the
polynucleotide with the nucleic acid according to claim 1.

44.   An antibody specifically reactive with a
polypeptide according to claim 27, claim 31 or claim 32.

45.   An antibody according to claim 44, wherein said
antibody is a polyclonal antibody.

46. An antibody according to claim 44, wherein said antibody is a monoclonal antibody or a F(ab')$_2$, F(ab'), F(ab) fragment or chimeric antibody.

47. An antibody according to claim 45 or 46, wherein said antibody is labeled with a detectable label.

48. An antibody according to claim 47, wherein said detectable label is radioactive isotope.

49. A method of making a polypeptide according to claim 27, claim 31 or claim 32, said method comprising the steps of:
    culturing a host cell transformed or transfected with a vector comprising a polynucleotide encoding said polypeptide operably associated with a promoter sequence such that the nucleic acid sequence encoding said polypeptide is expressed; and
    isolating said polypeptide from said host cell or from a growth medium in which said host cell is cultured.

50. A method of stimulating growth of connective tissue or wound healing in a mammal, comprising administering to said mammal an effective amount of the polypeptide according to claim 27, claim 31 or 32.

51. A method for producing an active truncated form of PDGF-C, comprising the steps of expressing an expression vector comprising a polynucleotide encoding a polypeptide as claimed in claim 69.

52. A method for regulating receptor-binding specificity of PDGF-C, comprising the steps of expressing

an expression vector comprising a polynucleotide encoding a polypeptide as claimed in claim 27. claim 31 or claim 32 and supplying a proteolytic amount of at least one enzyme for processing the expressed polypeptide to generate the active truncated form of PDGF-C.

53.    A method for selectively activating a polypeptide having a growth factor activity comprising the step expressing an expression vector comprising a polynucleotide encoding a polypeptide having a growth factor activity, a CUB domain and a proteolytic site between the polypeptide and the CUB domain, and supplying a proteolytic amount of at least one enzyme for processing the expressed polypeptide to generate the active polypeptide having a growth factor activity.

54.    An isolated polypeptide according to claim 27, claim 31 or claim 32 which comprises a proteolytic site having the amino acid sequence RKSR or a structurally conserved amino acid sequence thereof.

55.    An isolated nucleic acid molecule according to claim 10 which codes for a polypeptide which comprises a proteolytic site having the amino acid sequence RKSR or a structurally conserved amino acid sequence thereof.

56.    An isolated heterodimer comprising an active monomer of VEGF, VEGF-B, VEGF-C, VEGF-D, PDGF-C, PDGF-A, PDGF-B or PlGF and an active monomer of PDGF-C linked to a CUB domain.

57.    An isolated heterodimer comprising an active monomer of PDGF-C and an activated monomer of VEGF, VEGF-B,

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VEGF-C, VEGF-D, PDGF-C, PDGF-A, PDGF-B or P1GF linked to a
CUB domain.

58.   An isolated heterodimer according to claim 56,
further comprising a proteolytic site between the active
monomer and the CUB domain linkage.

59.   An isolated heterodimer according to claim 57,
further comprising a proteolytic site between the active
monomer and the CUB domain linkage.

60.   An isolated polynucleotide, comprising a
polynucleotide sequence having at least 85% identity with
the sequence of Figure 1, 3 or 5 (SEQ ID NOs: 2, 4 or 6,
respectively), or a polynucleotide which hybridize under
stringent conditions with at least one of said DNA
sequences.

61.   A method of promoting fibroblast mitogenesis in a
mammal, comprising the step of administering to said mammal
an effective fibroblast mitogenesis promoting amount of the
polypeptide according to claim 27, claim 31 or claim 32.

62.   A method of inducing PDGF alpha receptor
activation, comprising the step of adding a PDGF alpha-
receptor stimulating amount of the polypeptide according to
claim 27, claim 31 or claim 32.

63.   A method of inhibiting tumor growth of a tumor
expressing PDGF-C in a mammal, comprising administering to
said mammal a PDGF-C inhibiting amount of a PDGF-C
antagonist.

64.   A method of identifying specific types of human tumors, comprising the step of taking a sample of the tumor and testing for the expression of PDGF-C.

65.   The method of claim 64, wherein the specific types of tumors are selected from the group selected from choriocarcinoma, Wilms tumor, megakaryoblastic leukemia, lung carcinoma and erythroleukemia.

66.   A method for identifying an PDGF-C antagonist comprising:

admixing a substantially purified preparation of an activated truncated form of PDGF-C with a test agent; and

monitoring, by any suitable means, an inhibition in the biological activity of PDGF-C.

67.   A method for identifying an PDGF-C antagonist comprising:

admixing a substantially purified preparation of an full-length PDGF-C with a test agent; and

monitoring, by any suitable means, an inhibition in the cleavage of the CUB domain from PDGF-C.

68.   An isolated polypeptide according to claim 27, wherein the cells are selected from the group consisting of endothelial cells, connective tissue cells, myofibroblasts and glial cells.

69.   A method of making a vector which expresses a polypeptide comprising an amino acid sequence having at least 85% identity with the amino acid residues 230 to 345 of Figure 2 (SEQ ID NO:3) or of Figure 6 (SEQ ID NO:7),

said method comprising incorporating an isolated nucleic
acid molecule encoding said amino acid residues into said
vector in operatively linked relation with a promoter.

70.   An antibody according to claim 46, wherein said
monoclonal antibody is a humanized antibody.

71.   A method for producing an activated truncated
form of PDGF-C, comprising the steps of expressing an
expression vector comprising a polynucleotide encoding a
polypeptide as claimed in claim 27, claim 31 or claim 32
and supplying a proteolytic amount of at least one enzyme
for processing the expressed polypeptide to generate the
activated truncated form of PDGF-C.

72.   A method of inhibiting tissue remodeling during
invasion of tumor cells into a normal population of cells,
comprising administering to said mammal a PDGF-C inhibiting
amount of a PDGF-C antagonist.

73.   A method of treating fibrotic conditions in a
mammal in need a such treatment, comprising administering
to said mammal a PDGF-C inhibiting amount of a PDGF-C
antagonist.

74.   A method of claim 73, wherein the fibrotic
conditions are found in the lung, kidney or liver.

# Fig. 1

```
ccccgccgtg agtgagctct caccccagtc agccaaatga gcctcttcgg gcttctcctg 60

gtgacatctg ccctggccgg ccagagacga gggactcagg cggaatccaa cctgagtagt 120

aaattccagt tttccagcaa caaggaacag aacggagtac aagatcctca gcatgagaga 180

attattactg tgtctactaa tggaagtatt cacagcccaa ggtttcctca tacttatcca 240

agaaatacgg tcttggtatg gagattagta gcagtagagg aaaatgtatg gatacaactt 300

acgtttgatg aaagatttgg gcttgaagac ccagaagatg acatatgcaa gtatgatttt 360

gtagaagttg aggaacccag tgatggaact atattagggc gctggtgtgg ttctggtact 420

gtaccaggaa aacagatttc taaaggaaat caaattagga taagatttgt atctgatgaa 480

tattttcctt ctgaaccagg gttctgcatc cactacaaca ttgtcatgcc acaattcaca 540

gaagctgtga gtccttcagt gctacccct tcagctttgc cactggacct gcttaataat 600

gctataactg cctttagtac cttggaagac cttattcgat atcttgaacc agagagatgg 660

cagttggact tagaagatct atataggcca acttggcaac ttcttggcaa ggcttttgtt 720

tttggaagaa aatccagagt ggtggatctg aaccttctaa cagaggaggt aagattatac 780

agctgcacac tcgtaactt ctcagtgtcc ataagggaag aactaaagag aaccgatacc 840

attttctggc caggttgtct cctggttaaa cgctgtggtg ggaactgtgc ctgttgtctc 900

cacaattgca atgaatgtca atgtgtccca agcaaagtta ctaaaaaata ccacgaggtc 960

cttcagttga gaccaaagac cggtgtcagg ggattgcaca atcactcac cgacgtggcc 1020

ctggagcacc atgaggagtg tgactgtgtg tgcagaggga gcacaggagg atagccgcat 1080

caccaccagc agctcttgcc cagagctgtg cagtgcagtg gctgattcta ttagagaacg 1140

tatgcgttat ctccatcctt aatctcagtt gtttgcttca aggacctttc atcttcagga 1200
```

# Fig. 1 cont.

```
tttacagtgc attctgaaag aggagacatc aaacagaatt aggagttgtg caacagctct 1260

tttgagagga ggcctaaagg acaggagaaa aggtcttcaa tcgtggaaag aaaattaaat 1320

gttgtattaa atagatcacc agctagtttc agagttacca tgtacgtatt ccactagctg 1380

ggttctgtat ttcagttctt tcgatacggc ttagggtaat gtcagtacag gaaaaaaact 1440

gtgcaagtga gcacctgatt ccgttgcctt gcttaactct aaagctccat gtcctgggcc 1500

taaaatcgta taaaatctgg attttttttt tttttttttgc tcatattcac atatgtaaac 1560

cagaacattc tatgtactac aaacctggtt tttaaaaagg aactatgttg ctatgaatta 1620

aacttgtgtc rtgctgatag gacagactgg attttcata tttcttatta aaatttctgc 1680

catttagaag aagagaacta cattcatggt ttggaagaga taaacctgaa aagaagagtg 1740

gccttatctt cactttatcg ataagtcagt ttatttgttt cattgtgtac atttttatat 1800

tctcctttg acattataac tgttggcttt tctaatcttg ttaaatatat ctattttac 1860

caaaggtatt taatattctt ttttatgaca acttagatca actattttta gcttggtaaa 1920

tttttctaaa cacaattgtt atagccagag gaacaaagat ggatataaaa atattgttgc 1980

cctggacaaa aatacatgta tntccatccc ggaatggtgc tagagttgga ttaaacctgc 2040

attttaaaaa acctgaattg ggaanggaan ttggtaaggt tggccaaanc ttttttgaaa 2100

ataattaa                                                        2108
```

# Fig. 2

```
Met Ser Leu Phe Gly Leu Leu Leu Val Thr Ser Ala Leu Ala Gly Gln
 1               5                 10                15

Arg Arg Gly Thr Gln Ala Glu Ser Asn Leu Ser Ser Lys Phe Gln Phe
            20            25                30

Ser Ser Asn Lys Glu Gln Asn Gly Val Gln Asp Pro Gln His Glu Arg
        35            40                45

Ile Ile Thr Val Ser Thr Asn Gly Ser Ile His Ser Pro Arg Phe Pro
     50            55                60

His Thr Tyr Pro Arg Asn Thr Val Leu Val Trp Arg Leu Val Ala Val
65            70            75                80

Glu Glu Asn Val Trp Ile Gln Leu Thr Phe Asp Glu Arg Phe Gly Leu
            85            90                95

Glu Asp Pro Glu Asp Asp Ile Cys Lys Tyr Asp Phe Val Glu Val Glu
        100            105            110

Glu Pro Ser Asp Gly Thr Ile Leu Gly Arg Trp Cys Gly Ser Gly Thr
        115            120            125

Val Pro Gly Lys Gln Ile Ser Lys Gly Asn Gln Ile Arg Ile Arg Phe
    130            135                140

Val Ser Asp Glu Tyr Phe Pro Ser Glu Pro Gly Phe Cys Ile His Tyr
145            150            155                160

Asn Ile Val Met Pro Gln Phe Thr Glu Ala Val Ser Pro Ser Val Leu
            165            170                175

Pro Pro Ser Ala Leu Pro Leu Asp Leu Leu Asn Asn Ala Ile Thr Ala
        180            185            190

Phe Ser Thr Leu Glu Asp Leu Ile Arg Tyr Leu Glu Pro Glu Arg Trp
        195            200            205

Gln Leu Asp Leu Glu Asp Leu Tyr Arg Pro Thr Trp Gln Leu Leu Gly
    210            215            220

Lys Ala Phe Val Phe Gly Arg Lys Ser Arg Val Val Asp Leu Asn Leu
225            230            235                240

Leu Thr Glu Glu Val Arg Leu Tyr Ser Cys Thr Pro Arg Asn Phe Ser
            245            250            255

Val Ser Ile Arg Glu Glu Leu Lys Arg Thr Asp Thr Ile Phe Trp Pro
            260            265            270

Gly Cys Leu Leu Val Lys Arg Cys Gly Gly Asn Cys Ala Cys Cys Leu
        275            280            285
```

# Fig. 2 cont.

```
His Asn Cys Asn Glu Cys Gln Cys Val Pro Ser Lys Val Thr Lys Lys
    290                 295             300


Tyr His Glu Val Leu Gln Leu Arg Pro Lys Thr Gly Val Arg Gly Leu
305             310             315                 320

His Lys Ser Leu Thr Asp Val Ala Leu Glu His His Glu Glu Cys Asp
            325             330                 335

Cys Val Cys Arg Gly Ser Thr Gly Gly
            340             345
```

## Fig. 3

```
cgggtaaatt ccagttttcc agcaacaagg aacagaacgg agtacaagat cctcagcatg  60

agagaattat tactgtgtct actaatggaa gtattcacag cccaaggttt cctcatactt 120

atccaagaaa tacggtcttg gtatggagat tagtagcagt agaggaaaat gtatggatac 180

aacttacgtt tgatgaaaga tttgggcttg aagacccaga agatgacata tgcaagtatg 240

attttgtaga agttgaggaa cccagtgatg gaactatatt agggcgctgg tgtggttctg 300

gtactgtacc aggaaaacag atttctaaag gaaatcaaat taggataaga tttgtatctg 360

atgaatattt tccttctgaa ccagggttct gcatccacta caacattgtc atgccacaat 420

tcacagaagc tgtgagtcct tcagtgctac ccccttcagc tttgccactg gacctgctta 480

ataatgctat aactgccttt agtaccttgg aagaccttat tcgatatctt gaaccagaga 540

gatggcagtt ggacttagaa gatctatata ggccaacttg gcaacttctt ggcaaggctt 600

ttgtttttgg aagaaaatcc agagtggtgg atctgaacct tctaacagag gaggtaagat 660

tatacagctg cacacctcgt aacttctcag tgtccataag ggaagaacta aagagaaccg 720

ataccatttt ctggccaggt tgtctcctgg ttaaacgctg tggtgggaac tgtgcctgtt 780

gtctccacaa ttgcaatgaa tgtcaatgtg tcccaagcaa agttactaaa aaataccacg 840

aggtccttca gttgagacca aasaccggtg tcaggggatt gcacaaatca ctcaccgacg 900

tggccctgga gcaccatgag gagtgtgact gtgtgtgcag agggagcaca ggaggatagc 960

cgcatcacca ccagcagctc ttgcccagag ctgtgcagtg cagtggctga ttctattaga 1020

gaacgtatgc gttatctcca tccttaatct cagttgtttg cttcaaggac ctttcatctt 1080

caggatttac agtgcattct gaaagaggag acatcaaaca gaattaggag ttgtgcaaca 1140

gctcttttga gaggaggcct aaaggacagg agaaaaggtc ttcaatcgtg gaaagaaaat 1200
```

# Fig. 3 cont.

```
taaatgttgt attaaataga tcaccagcta gtttcagagt taccatgtac gtattccact 1260

agctgggttc tgtatttcag ttctttcgat acggcttagg gtaatgtcag tacaggaaaa 1320

aaactgtgca agtgagcacc tgattccgtt gccttgctta actctaaagc tccatgtcct 1380

gggcctaaaa tcgtataaaa tctggatttt tttttttttt tttgctcata ttcacatatg 1440

taaaccagaa cattctatgt actacaaacc tggttttaa aaaggaacta tgttgctatg 1500

aattaaactt gtgtcatgct gataggacag actgga                            1536
```

# Fig. 4

```
Gly Lys Phe Gln Phe Ser Ser Asn Lys Glu Gln Asn Gly Val Gln Asp
 1               5                   10                  15

Pro Gln His Glu Arg Ile Ile Thr Val Ser Thr Asn Gly Ser Ile His
                20                  25                  30

Ser Pro Arg Phe Pro His Thr Tyr Pro Arg Asn Thr Val Leu Val Trp
        35                  40                  45

Arg Leu Val Ala Val Glu Glu Asn Val Trp Ile Gln Leu Thr Phe Asp
    50                  55                  60

Glu Arg Phe Gly Leu Glu Asp Pro Glu Asp Asp Ile Cys Lys Tyr Asp
65                  70                  75                  80

Phe Val Glu Val Glu Glu Pro Ser Asp Gly Thr Ile Leu Gly Arg Trp
                85                  90                  95

Cys Gly Ser Gly Thr Val Pro Gly Lys Gln Ile Ser Lys Gly Asn Gln
            100                 105                 110

Ile Arg Ile Arg Phe Val Ser Asp Glu Tyr Phe Pro Ser Glu Pro Gly
        115                 120                 125

Phe Cys Ile His Tyr Asn Ile Val Met Pro Gln Phe Thr Glu Ala Val
    130                 135                 140

Ser Pro Ser Val Leu Pro Pro Ser Ala Leu Pro Leu Asp Leu Leu Asn
145                 150                 155                 160

Asn Ala Ile Thr Ala Phe Ser Thr Leu Glu Asp Leu Ile Arg Tyr Leu
            165                 170                 175

Glu Pro Glu Arg Trp Gln Leu Asp Leu Glu Asp Leu Tyr Arg Pro Thr
            180                 185                 190

Trp Gln Leu Leu Gly Lys Ala Phe Val Phe Gly Arg Lys Ser Arg Val
        195                 200                 205

Val Asp Leu Asn Leu Leu Thr Glu Glu Val Arg Leu Tyr Ser Cys Thr
    210                 215                 220

Pro Arg Asn Phe Ser Val Ser Ile Arg Glu Glu Leu Lys Arg Thr Asp
225                 230                 235                 240

Thr Ile Phe Trp Pro Gly Cys Leu Leu Val Lys Arg Cys Gly Gly Asn
            245                 250                 255

Cys Ala Cys Cys Leu His Asn Cys Asn Glu Cys Gln Cys Val Pro Ser
            260                 265                 270

Lys Val Thr Lys Lys Tyr His Glu Val Leu Gln Leu Arg Pro Lys Thr
        275                 280                 285
```

## Fig. 4 cont.

```
Gly Val Arg Gly Leu His Lys Ser Leu Thr Asp Val Ala Leu Glu His
    290                 295             300

His Glu Glu Cys Asp Cys Val Cys Arg Gly Ser Thr Gly Gly
305             310             315
```

# Fig. 5

```
cacctggaga cacagaagag ggctctagga aaaattttgg atggggatta tgtggaaact  60

accctgcgat tctctgctgc cagagccggc caggcgcttc caccgcagcg cagcctttcc  120

ccgggctggg ctgagccttg gagtcgtcgc ttccccagtg cccgccgcga gtgagccctc  180

gccccagtca gccaaatgct cctcctcggc ctcctcctgc tgacatctgc cctggccggc  240

caaagaacgg ggactcgggc tgagtccaac ctgagcagca agttgcagct ctccagcgac  300

aaggaacaga acggagtgca agatccccgg catgagagag ttgtcactat atctggtaat  360

gggagcatcc acagcccgaa gtttcctcat acgtacccaa gaaatatggt gctggtgtgg  420

agattagttg cagtagatga aaatgtgcgg atccagctga catttgatga gagatttggg  480

ctggaagatc cagaagacga tatatgcaag tatgattttg tagaagttga ggagcccagt  540

gatggaagtg ttttaggacg ctggtgtggt tctgggactg tgccaggaaa gcagacttct  600

aaaggaaatc atatcaggat aagatttgta tctgatgagt attttccatc tgaacccgga  660

ttctgcatcc actacagtat tatcatgcca caagtcacag aaaccacgag tccttcggtg  720

ttgccccctt catctttgtc attggacctg ctcaacaatg ctgtgactgc cttcagtacc  780

ttggaagagc tgattcggta cctagagcca gatcgatggc aggtggactt ggacagcctc  840

tacaagccaa catggcagct tttgggcaag gctttcctgt atgggaaaaa aagcaaagtg  900

gtgaatctga atctcctcaa ggaagaggta aaactctaca gctgcacacc ccggaacttc  960

tcagtgtcca tacgggaaga gctaagagg acagatacca tattctggcc aggttgtctc  1020

ctggtcaagc gctgtggagg aaattgtgcc tgttgtctcc ataattgcaa tgaatgtcag  1080

tgtgtcccac gtaaagttac aaaaaagtac catgaggtcc ttcagttgag accaaaaact  1140

ggagtcaagg gattgcataa gtcactcact gatgtggctc tggaacacca cgaggaatgt  1200
```

# Fig. 5 cont.

```
gactgtgtgt gtagaggaaa cgcaggaggg taactgcagc cttcgtagca gcacacgtga 1260

gcactggcat tctgtgtacc cccacaagca accttcatcc ccaccagcgt tggccgcagg 1320

gctctcagct gctgatgctg gctatggtaa agatcttact cgtctccaac caaattctca 1380

gttgtttgct tcaatagcct tcccctgcag gacttcaagt gtcttctaaa agaccagagg 1440

caccaanagg agtcaatcac aaagcactgc accg                                1474
```

# Fig. 6

```
Met Leu Leu Leu Gly Leu Leu Leu Leu Thr Ser Ala Leu Ala Gly Gln
 1               5                   10              Ala         15

Arg Thr Gly Thr Arg Ala Glu Ser Asn Leu Ser Ser Lys Leu Gln Leu
         20                  25                  30

Ser Ser Asp Lys Glu Gln Asn Gly Val Gln Asp Pro Arg His Glu Arg
         35                  40                  45

Val Val Thr Ile Ser Gly Asn Gly Ser Ile His Ser Pro Lys Phe Pro
     50                  55                  60

His Thr Tyr Pro Arg Asn Met Val Leu Val Trp Arg Leu Val Ala Val
 65              70                  75                  80

Asp Glu Asn Val Arg Ile Gln Leu Thr Phe Asp Glu Arg Phe Gly Leu
             85                  90                  95

Glu Asp Pro Glu Asp Asp Ile Cys Lys Tyr Asp Phe Val Glu Val Glu
            100                 105                 110

Glu Pro Ser Asp Gly Ser Val Leu Gly Arg Trp Cys Gly Ser Gly Thr
            115                 120                 125

Val Pro Gly Lys Gln Thr Ser Lys Gly Asn His Ile Arg Ile Arg Phe
    130                 135                 140

Val Ser Asp Glu Tyr Phe Pro Ser Glu Pro Gly Phe Cys Ile His Tyr
145                 150                 155                 160

Ser Ile Ile Met Pro Gln Val Thr Glu Thr Thr Ser Pro Ser Val Leu
            165                 170                 175

Pro Pro Ser Ser Leu Ser Leu Asp Leu Leu Asn Asn Ala Val Thr Ala
            180                 185                 190

Phe Ser Thr Leu Glu Glu Leu Ile Arg Tyr Leu Glu Pro Asp Arg Trp
            195                 200                 205

Gln Val Asp Leu Asp Ser Leu Tyr Lys Pro Thr Trp Gln Leu Leu Gly
    210                 215                 220

Lys Ala Phe Leu Tyr Gly Lys Lys Ser Lys Val Val Asn Leu Asn Leu
225                 230                 235                 240

Leu Lys Glu Glu Val Lys Leu Tyr Ser Cys Thr Pro Arg Asn Phe Ser
            245                 250                 255

Val Ser Ile Arg Glu Glu Leu Lys Arg Thr Asp Thr Ile Phe Trp Pro
            260                 265                 270

Gly Cys Leu Leu Val Lys Arg Cys Gly Gly Asn Cys Ala Cys Cys Leu
            275                 280                 285
```

# Fig. 6 cont.

```
His Asn Cys Asn Glu Cys Gln Cys Val Pro Arg Lys Val Thr Lys Lys
    290             295         300
```

```
Tyr His Glu Val Leu Gln Leu Arg Pro Lys Thr Gly Val Lys Gly Leu
305             310         315             320
```

```
His Lys Ser Leu Thr Asp Val Ala Leu Glu His His Glu Glu Cys Asp
            325             330             335
```

```
Cys Val Cys Arg Gly Asn Ala Gly Gly
            340         345
```

## Fig. 7

```
hPDGF-C   M S L F G L L L V T S A L A G Q R R G T A E S N L S S K F Q F S S N K E Q N G   40
mPDGF-C   M L L L G I I I L T S A I A G Q R I G T R A F C H I S S F I F S Q F F Q N G   40

hPDGF-C   V Q D P Q H E R I I T V S T N G S I H S   D F P H T Y P R N T V L V W R L V A V   80
mPDGF-C   V Q D P R H F V V I I S G V G S I H S R F P H T V P R N M V L V W R L V A V   80

hPDGF-C   I E N V H I Q L T F D E R F G L E D P E D D I C K Y D F V E V E E P S D G T T I   120
mPDGF-C   D F N V R I Q L T F D E R F G L F D P F D D I C K V D F V F F P S D G S V S   120

hPDGF-C   G R W C G S G T V P G K Q I S K G N Q I R I R F V S D E Y F P S E P G F C I H T   160
mPDGF-C   G R W C G S G T V P G K Q T S K G N H I R I R F V S D F V F P S F P G F C I H S   160

hPDGF-C   N I V M P Q F T E A V S P S V L P P S A L D L D L L N N A I T A F S T L F D L I   200
mPDGF-C   S I I M D Q V I F T T S F V L P D S S L S L D L L N N B V T A I S T L F L   200

hPDGF-C   R Y L E P F R W Q I D L F D L Y R P T W Q L L G K A F V F G R K S R V V D L N I   240
mPDGF-C   S V I F D D F W D V D I D S L I V K D T W Q L L G I A S L V D R K S H V J N L N J   240

hPDGF-C   L T E E V R L Y S C T P R N F S V S I R E E L K R T D T I F W P G C L L V K R G   280
mPDGF-C   I K F F V K L Y S C T P R N F S V S I R F E L K R T D T I F W P G C L L V K R G   280

hPDGF-C   G G N C A C C L H N C N E C Q C V P S K V T K K Y H E V L Q L R P K T G V R G I   320
mPDGF-C   G G N C A C C L H N C N F C Q C V S R K V T K K V H F V L Q L R P K T G V R G I   320

hPDGF-C   H K S L T D V A L E H H E E C D C V C R G S T G G                                 345
mPDGF-C   H I S L T D V A L F H H F F C D C V C R G N A G G                                 345
```

## Fig. 8



signal sequence

CUB domain      PDGF/VEGF Homology Domain
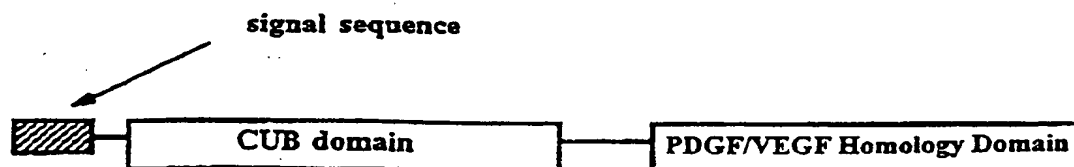
# Fig. 9

```
VEGF 165       - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   1
PlGF-2         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   1
VEGF-B167      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   1
Pox Orf VEGF   M H L L G F F S V A C S L L A A A L L P G P R E A P A A A A  30
VEGF-C         - - - - - - - - - - - - - - M Y G E W G M G N I L M M F H  15
VEGF-D         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   1
PDGF-A         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   1
PDGF-B         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   1
hPDGF-C PVDV   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   1
mPDGF-C PVDV   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   1


VEGF 165       - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   1
PlGF-2         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   1
VEGF-B167      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   1
Pox Orf VEGF   A F E S G L D L S D A E P D A G E A T A Y A S K D L E E Q L  60
VEGF-C         V Y L V Q G F R S E H G P V K D F S F E R S S R S M L E R S  45
VEGF-D         - - - - M R T L A C L L L L L G C G Y L A H V L A E E A E I P  26
PDGF-A         M N R C W A L F L S L C C Y L R L V S A E G D P I P E E L Y  30
PDGF-B         - - - - - - - M P Q F T E A V S P S V L P P S A L P L D L L  23
hPDGF-C PVDV   - - - - - - - M P Q V T E T T S P S V L P P S S L S L D L L  23


VEGF 165       - - - - - - - - - - - - - - - - - - - M N F L L S W V H W  10
PlGF-2         - - - - - - - - - - - - - - - - - - - M P V M R L F P C F  10
VEGF-B167      - - - - - - - - - - - - - - - - - - - - - - M S P L L  5
Pox Orf VEGF   R S V S S V D E L M T V L Y P E Y W K M Y K C Q L R K G G W  90
VEGF-C         E Q Q I R A A S S L E E L L Q I A H S E D W K L W R C R L K  75
VEGF-D         R E V I E R L A R S Q I H S I R D L Q R L L E I D S V G S E  56
PDGF-A         E M L S D H S I R S F D D L Q R L L H G D P - - - - - G E E  53
PDGF-B         N N A I T A F S T L E D L I R Y L E P E R W Q L D L E D L Y  53
hPDGF-C PVDV   N N A V T A F S T L E E L I R Y L E P D R W Q V D L D S L L Y  53


VEGF 165       S L A F L L Y L H H A K W S Q A A P M A E G G G Q N H H E V  40
PlGF-2         T Q L L A G L A L P A V P P Q Q W A L S A G N G S S E V E V  40
VEGF-B167      R R L L A A L L Q L A P A Q A P V S Q P D A P G H Q R K V  35
Pox Orf VEGF   - - - - - M K L L V G I L V A V C L H Q Y L L N A D S N T  24
VEGF-C         Q H N R E Q A N L N S R T E E T I K F A A A H Y N T E I - L  119
VEGF-D         L K S L A S M D S R S A S H R S T R F A A T F Y D T E T - L  104
PDGF-A         D S L D T S L R A H G V H - - A T K H V P E K R P L P I R R  84
PDGF-B         D G A E L D L N M T R S H S G G E L E S L A R G R R S L G S  85
hPDGF-C PVDV   R P T W Q L L G K A F V F G R K S R - - - - - - - - V V D L  75
mPDGF-C PVDV   K P T W Q L L G K A F L Y G K K S K - - - - - - - - V V N L  75


VEGF 165       V K F M D V Y Q R S Y C H P I E T L V D I F Q E Y P D E I E  70
PlGF-2         V P F Q E V W G R S Y C R A L E R L V D V S E Y P S E V E  70
VEGF-B167      V S W I D V Y T R A T C Q P R E V V P L T V E L M G T V A  65
Pox Orf VEGF   K G W S E V L K G S E C K P R P I V V P V S E T H P E L T S  54
VEGF-C         K S I D N E W R K T Q C M P R E V C I D V G K E F G V A T N  149
VEGF-D         K V I D E E W Q R T Q C S P R E T C V E V A S E L G K T T N  134
PDGF-A         K R S I E E A V P A V C K T R T V I Y E I P R S Q V D P T S  114
PDGF-B         L T I A E P A M I A E C K T R T E V F E I S R R L I D R T N  115
hPDGF-C PVDV   N L L T E E V R L Y S C T P R N F S V S I - R E E L K R T D  104
mPDGF-C PVDV   N L L K E E V K L Y S C T P R N F S V S I - R E E L K R T D  104
```

# Fig. 9 cont.

```
VEGF 165       Y I F K - - P S C V P L M R C G G - - - C C N D E G L E C V   95
PlGF-2         H M E S - - P S C V S L L R C T G - - - C C G D E D L H C V   95
VEGF-B167      K Q L V - - P S C V T V Q R C G G - - - C C P D D G L E C V   90
Pox Orf VEGF   Q R F N - - P P C V T L M R C G G - - - C C N D E S L E C V   79
VEGF-C         T F F K - - P P C V S V Y R C G G - - - C C N S E G L Q C M   174
VEGF-D         T F F K - - P P C V N V F R C G G - - - C C N E E G V M C M   159
PDGF-A         A N F L I W P P C V E V K R C T G - - - C C N T S S V K C Q   141
PDGF-B         A N F L V W P P C V E V Q R C S G - - - C C N N R N V Q C R   142
hPDGF-C PVDV   T I F - - W P G C L L V K R C G G N C A C C L H N C N E C Q   132
mPDGF-C PVDV   T I F - - W P G C L L V K R C G G N C A C C L E N C N E C Q   132

VEGF 165       P T E E S N I T M Q I M R I K - - - P H Q G Q - - - - - - H I   117
PlGF-2         P V E T A N V T M Q L L K I R - - - S G D R P - - - - - - S Y   117
VEGF-B167      P T G Q H Q V R M Q I L M I R Y - - P S S Q L - - - - - - R L   111
Pox Orf VEGF   P T E E V N V S M E L L G A S G S G S N G M Q - - - - - P K   197
VEGF-C         N T S T S Y L S K T L F E I T V - - P L S Q G - - - - - P E   182
VEGF-D         N T S T S Y I S K Q L F E I S V - - P L T S V - - - - - P E   166
PDGF-A         P S R V H H R S V K V A K V E Y V R K K P K L - - - - - K E   166
PDGF-B         P T Q V Q L R P V Q V R K I E I V R K K P I F - - - - - K K   167
hPDGF-C PVDV   C V P - S K V T K K Y H E V L Q L R P K T G V R G L H K S L   161
mPDGF-C PVDV   C V P - R K V T K K Y H E V L Q L R P K T G V K G L H K S L   161

VEGF 165       G E M S F L Q H N K - C E C R P K K - - - - - - - - - - D R   136
PlGF-2         V E L T F S Q H V R - C E C R P L R E - - - - K M K P E R R   142
VEGF-B167      G E M S L E E H S Q - C E C R P K K K - - - - - D S A V K P   135
Pox Orf VEGF   S F V E H K K - - - - C D C R P R F T - - - - - - - T T P P   123
VEGF-C         P V T I S F A N H T S C R C M S K L D - - - V Y R Q V H S I   224
VEGF-D         L V P V K I A N H T G C K C L P T G P - - - - - R H P Y S I   207
PDGF-A         V Q V R L E E H L E - C A C A T T S L N P D Y R E E D T G R   195
PDGF-B         A T V T L E D H L A - C K C E T V A A A R P V T R S P G G S   196
hPDGF-C PVDV   T D V A L E H H E E - C D C V C R G S T G G                   182
mPDGF-C PVDV   T D V A L E H H E E - C D C V C R G N A G G                   182

VEGF 165       A R Q E N P C G P C S S E R R K H L F V Q D P Q T C K C S C   166
PlGF-2         R P K G R G K R R R E N Q R P T D C H L C G D A V P R R       170
VEGF-B167      D S P R P L C E R C T Q H H Q R P D P R T - - - - C R C R C   161
Pox Orf VEGF   T T T R P P R R R R                                           133
VEGF-C         I R R S L P A T - L P Q C Q A A N K T C P T N Y M W N N H I   253
VEGF-D         I R R S I Q T P E E D E C P H S K K L C P I D M L W D N T K   237
PDGF-A         P R E S G K K R K R K R L K P T                               211
PDGF-B         Q E Q R A K T P Q T R V T I R T V R V R R P P K G K H R K F   225
hPDGF-C PVDV                                                                182
mPDGF-C PVDV                                                                182

VEGF 165       K N T D S - R C K A R Q L E L N E R T C R C D K P R R         192
PlGF-2                                                                      170
VEGF-B167      R R R S F L R C Q G R G L E L N P D T C R C R K L R R         188
Pox Orf VEGF                                                                133
VEGF-C         C R C L A Q E D F M F S S D A G D D S T D G F H D I C G P N   283
VEGF-D         C K C V L Q D E - T P L P G T E D H S Y L Q E P T L C G P H   266
PDGF-A                                                                      211
PDGF-B         K H T H D K T A L K E T L G A                                241
hPDGF-C PVDV                                                                182
mPDGF-C PVDV                                                                182
```

# Fig. 9 cont.

```
VEGF 165                                                                          192
P1GF-2                                                                            170
VEGF-B167                                                                         188
Pox Orf VEGF                                                                      133
VEGF-C         K E L D E E T C Q C V C R A G L R P A S C G P H K E L D R N       313
VEGF-D         M T F D E D R - - - - - - - - - - - - - - - - - - - - -          273
PDGF-A                                                                           211
PDGF-B                                                                           241
hPDGF-C PVDV                                                                      182
mPDGF-C PVDV                                                                      182


VEGF 165                                                                          192
P1GF-2                                                                            170
VEGF-B167                                                                         188
Pox Orf VEGF                                                                      133
VEGF-C         S C Q C V C R N K L F P S Q C G A N R E F D E N T C Q C V C       343
VEGF-D         - C E C V C K A P C P G D L I Q H P E N - - - - - C S C F E       297
PDGF-A                                                                           211
PDGF-B                                                                           241
hPDGF-C PVDV                                                                      182
mPDGF-C PVDV                                                                      182


VEGF 165                                                                          192
P1GF-2                                                                            170
VEGF-B167                                                                         188
Pox Orf VEGF                                                                      133
VEGF-C         K R T C P R N Q P L N P G K C A C E C T E S P Q K C L L K G       373
VEGF-D         C K E S L E S C C Q K H K I - - - - - - - - - - - - - - -         312
PDGF-A                                                                           211
PDGF-B                                                                           241
hPDGF-C PVDV                                                                      182
mPDGF-C PVDV                                                                      182


VEGF 165                                                                          192
P1GF-2                                                                            170
VEGF-B167                                                                         188
Pox Orf VEGF                                                                      133
VEGF-C         K K F H H Q T C S C Y R R P C T N R Q K A C E P G F S Y S E       403
VEGF-D         - - F H P D T C S C E D R C P F H T R T C A S R K P A C G         338
PDGF-A                                                                           211
PDGF-B                                                                           241
hPDGF-C PVDV                                                                      182
mPDGF-C PVDV                                                                      182


VEGF 165                                                                          192
P1GF-2                                                                            170
VEGF-B167                                                                         188
Pox Orf VEGF                                                                      133
VEGF-C         E V C R C V P S Y W K R P Q M S                                    419
VEGF-D         K H W R F P K E T R A Q G L Y S Q E N P                            358
PDGF-A                                                                           211
PDGF-B                                                                           241
hPDGF-C PVDV                                                                      182
mPDGF-C PVDV                                                                      182
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Fig. 10
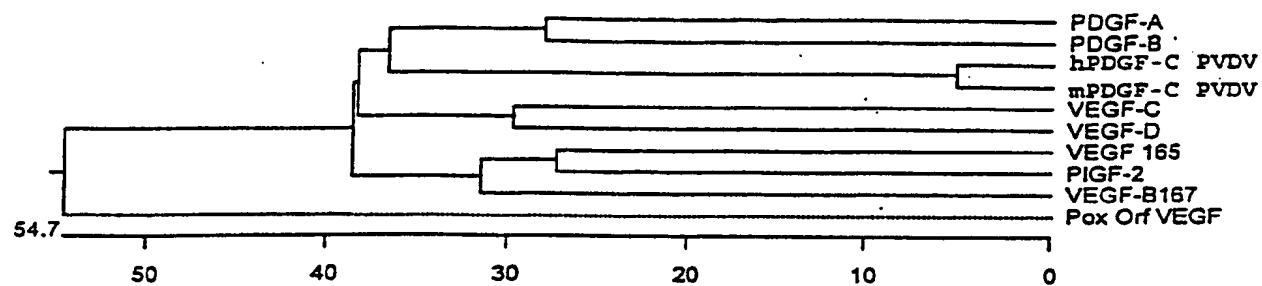


Amino acid replacements (per 100 residues)

## Fig. 11



```
mPDGF-C CUB   E R V V T I S G N G S I H S P K F P H T Y P R N M V L V W R L V A V D E N V R I   86
hPDGF-C CUB   G R I I T V S T N G S I H S P R P P H I Y P R N T V L V W R L V A V E E N V W I   59
hBMP-1 CUB1   C G E T L Q D S T G N F S S P E Y P N G I S A H M H C V W R I S V T P G E - K I   360
hBMP-1 CUB2   C G G D V K K D Y G H I Q S P N Y P D D Y R P S K V C I W R I O V S E G F - H V   473
hBMP-1 CUB3   C G G F L T K L N G S I T S P G W P K E Y P P R K N C I W Q L V A P T Q Y - R I   629
Neuropilin CUB1  G D T I K E S P G Y L T S P G Y P H S Y H P S E K C E W L I Q A P D P Y Q R I   67
Neuropilin CUB2  C S Q N Y T T P S G V I K S G F E K Y P W S L E C T Y I V P A P K M S E - I   193

mPDGF-C CUB   Q L T F D E R F G L E D - - - - P E D D I C K Y D P V E V E E - - P S D G S V L   120
hPDGF-C CUB   Q L T F D E R F G L E D - - - - P E D D I C K Y D P V E V E E - - P S D G T I L   93
hBMP-1 CUB1   I L N F T S - L D L Y R S R - - - - - - L C W Y D Y V E V R D G P W R K A P L R   393
hBMP-1 CUB2   G L T F Q S - F E I E R K D - - - - - - S C A Y D Y L E V R D G H S E S S T L I   506
hBMP-1 CUB3   S L Q F D F - F E T E G N D - - - - - - V C K Y D F V E V R S G L T A D S K L H   662
Neuropilin CUB1  M I N F N P H T D L E D R D - - - - - - C K Y D Y V E V F D G E N E N G H F R   100
Neuropilin CUB2  I I E E S - F D L E P D S N P G C M F C R Y D R L E I W D G F P D V G P H I   224

mPDGF-C CUB   G R W C G S G T V P G K Q T S K G N K I R I R F V S D E Y F P S E P G F C I H Y   160
hPDGF-C CUB   G R Y C G S G T V P G K Q I S K G N Q I R I R F V S D E Y F P S E P G P C I H Y   133
hBMP-1 CUB1   G R F C G S - K L P E P I V S T D S R L W V E F R S S S N W V G K - G F F A V Y   431
hBMP-1 CUB2   G R Y C G Y - E K P D D I K S T S S R L W L K F V S D G S I N K A - G F A V N F   544
hBMP-1 CUB3   G K F C G S - E K P E V I T S Q Y R N H R V E F K S D N T V S K K - G F K A H F   700
Neuropilin CUB1  G K F C G K - I A P P P V V S S G P F L F T K F V S D Y E T H G A - G F S I R Y   138
Neuropilin CUB2  G R Y C G Q - K T P G R I R S S G I L S H V F Y T D S A I A K E - G F S A N Y   262

mPDGF-C CUB   S I I                                                                        163
hPDGF-C CUB   N I V                                                                        136
hBMP-1 CUB1   E A T                                                                        424
hBMP-1 CUB2   F K                                                                          546
hBMP-1 CUB3   F S E                                                                        703
Neuropilin CUB1  - E T                                                                       140
Neuropilin CUB2  S V L                                                                       265
```

## Fig. 12

## Fig. 13



## Fig. 14

## Fig. 15



## Fig. 18



PDGF BB (ng/ml)

## Fig. 19



PDGF-C protein (ng/ml)
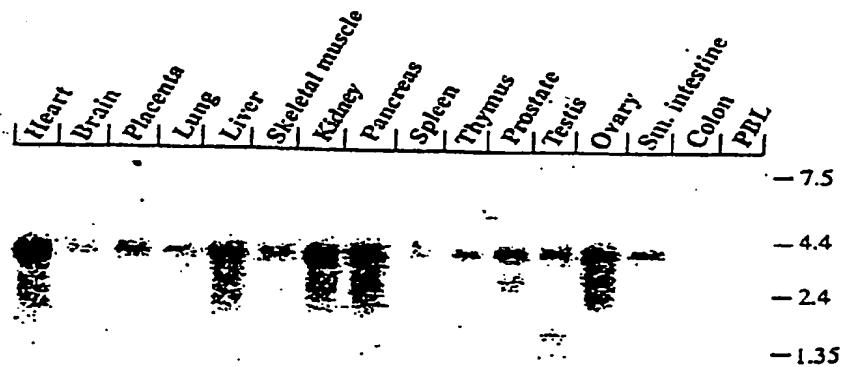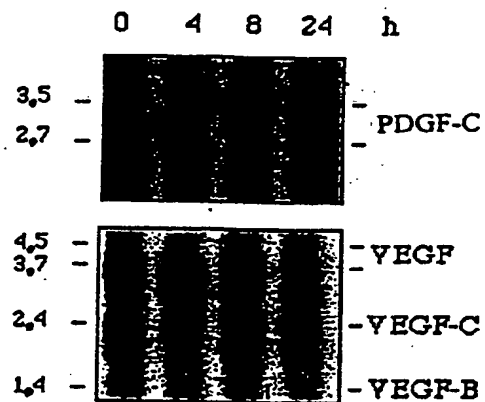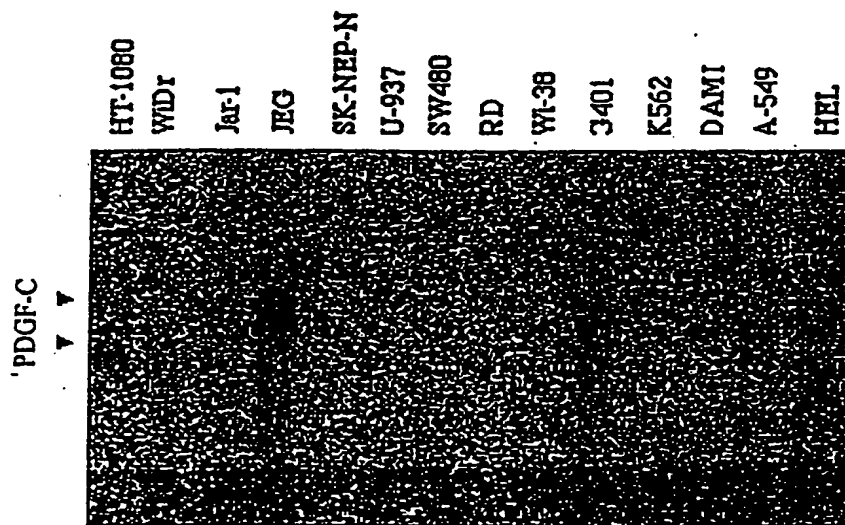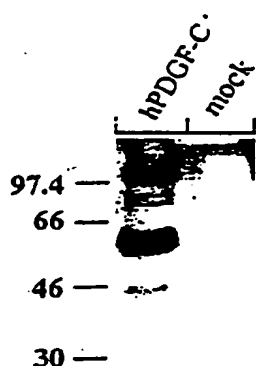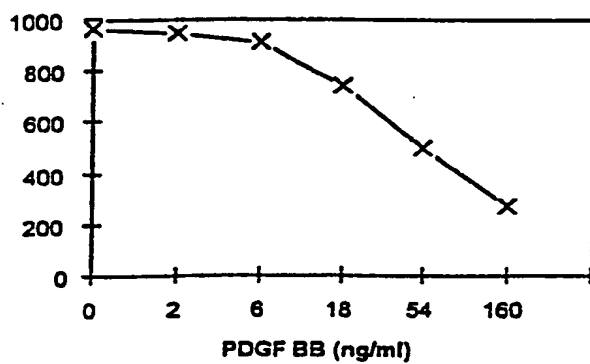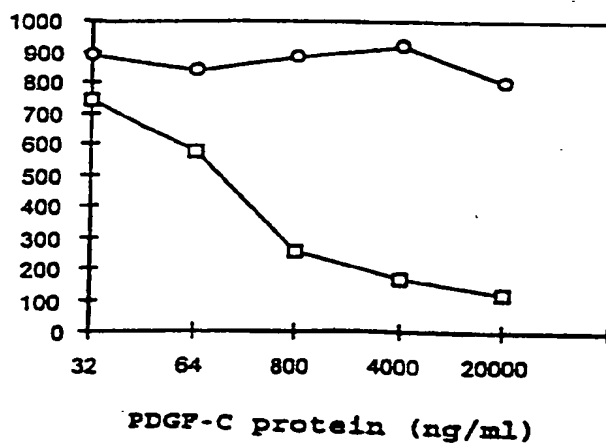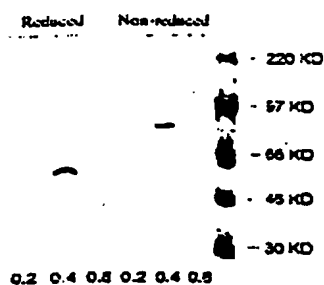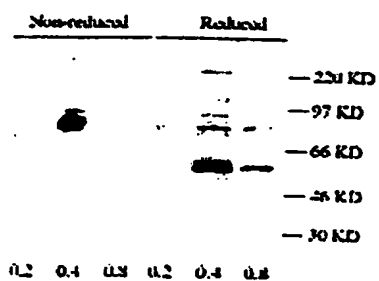
## Fig. 16A



## Fig. 16B



## Fig. 16C
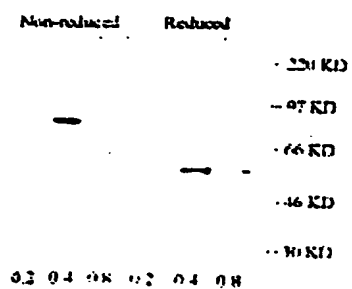
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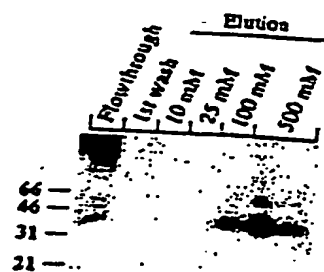


## Fig. 17B
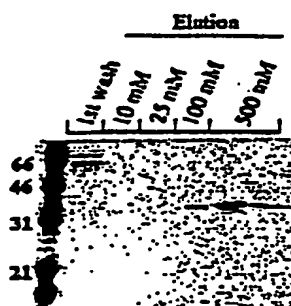


## Fig. 17C

Fig. 20
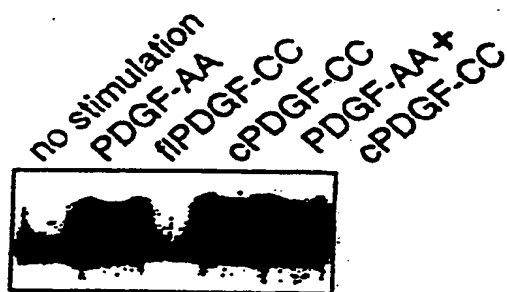


IP: PDGF alpha-rec.
IB: P-Tyr

Fig. 21
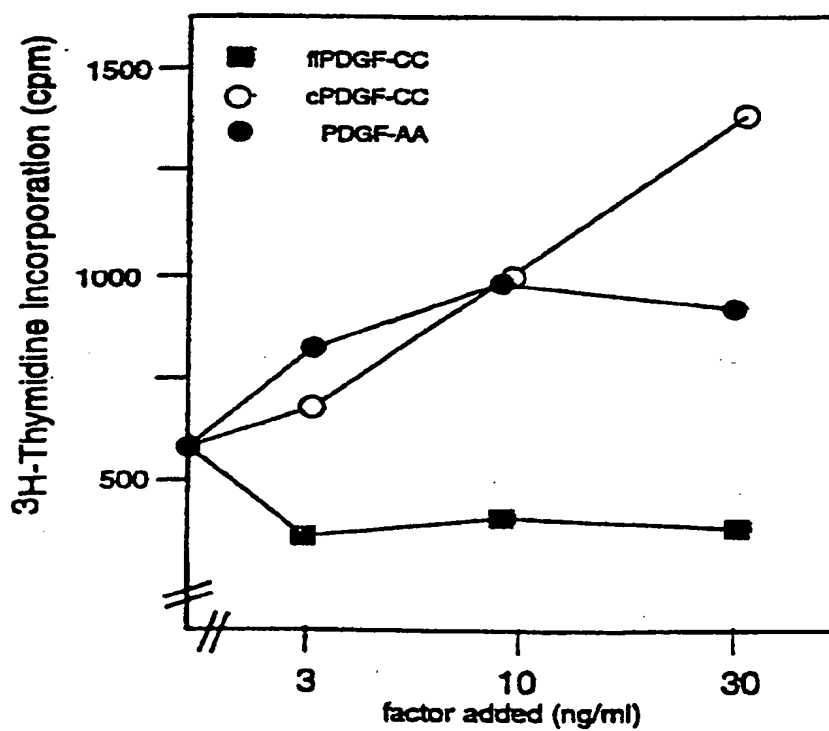
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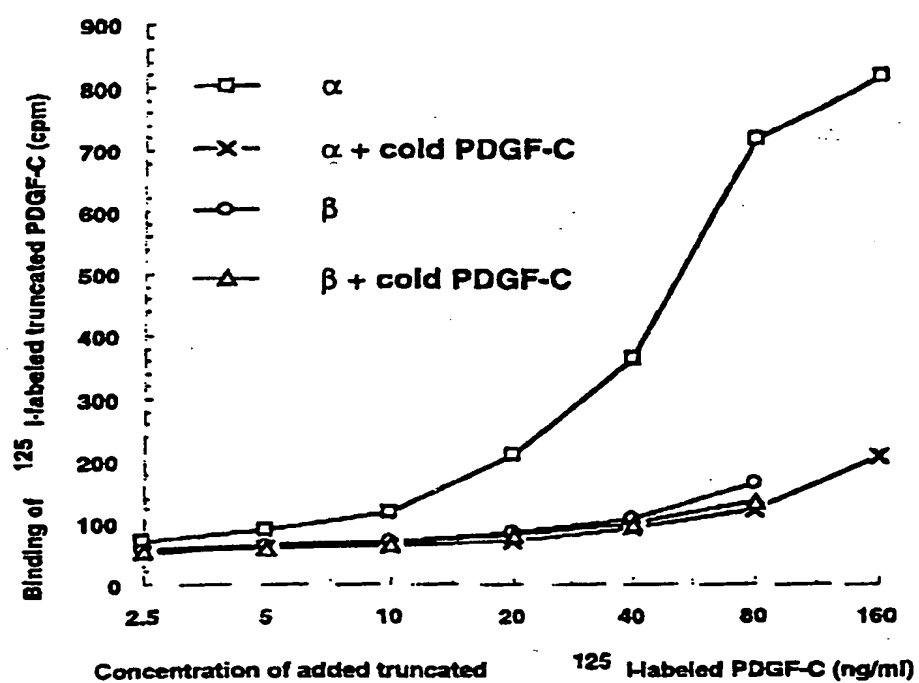
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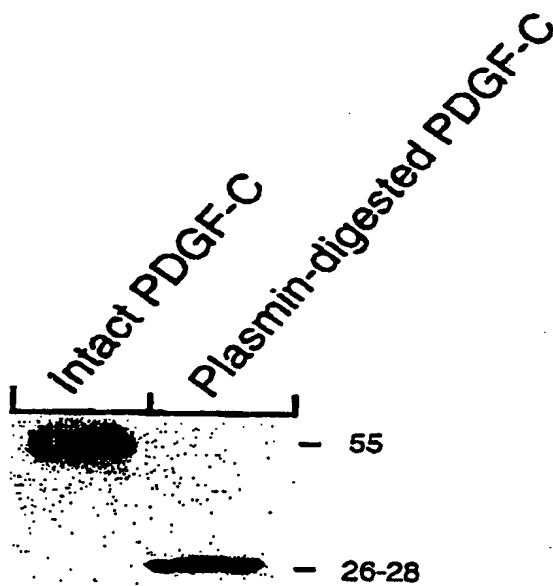
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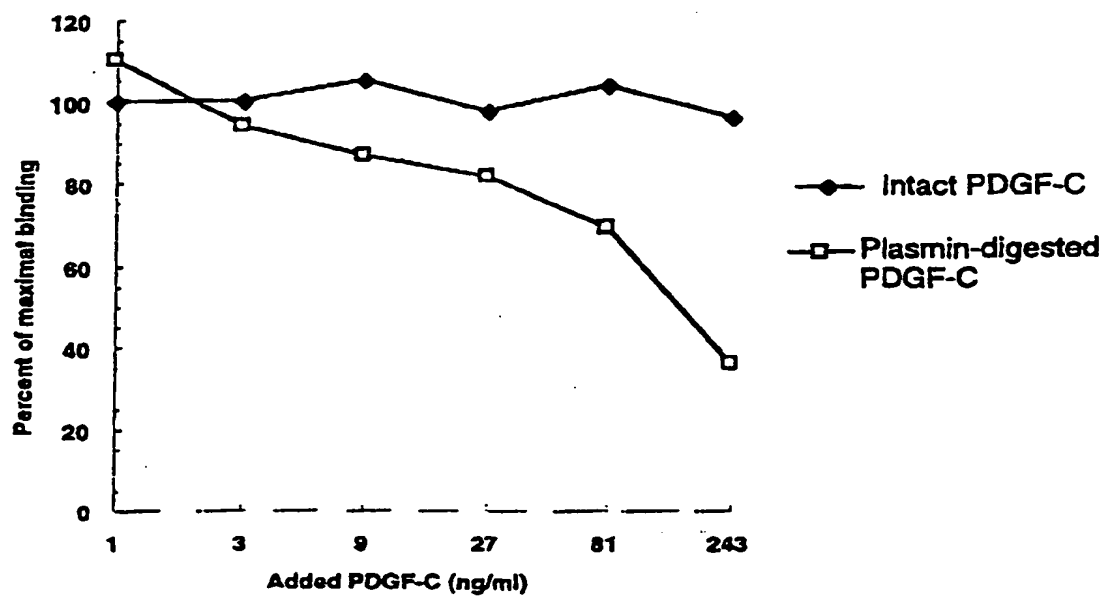
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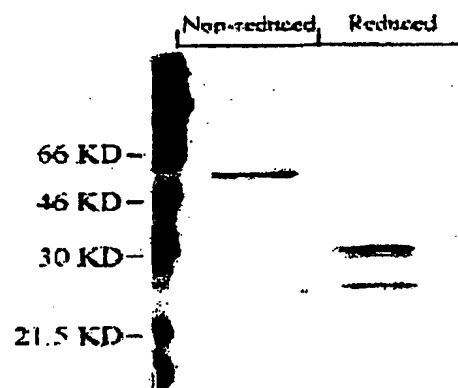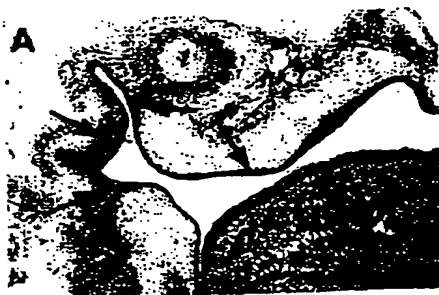
Fig. 26A          Fig. 26B          Fig. 26C

Fig. 26D          Fig. 26E          Fig. 26F
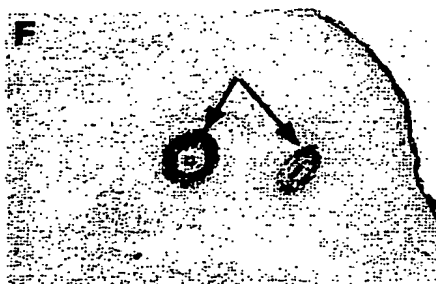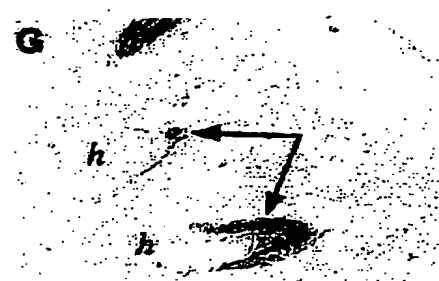
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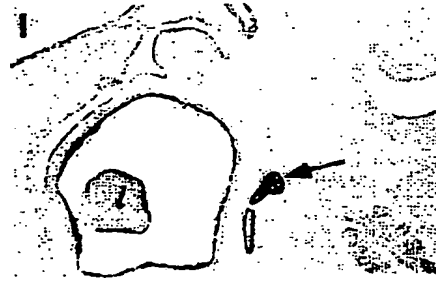
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Fig. 26M      Fig. 26N      Fig. 26O

Fig. 26P         Fig. 26Q
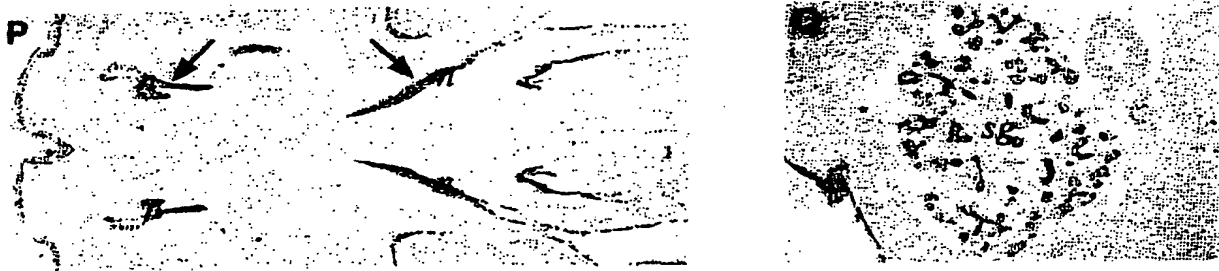
Fig. 26R        Fig. 26S

Fig. 26T      Fig. 26U      Fig. 26V
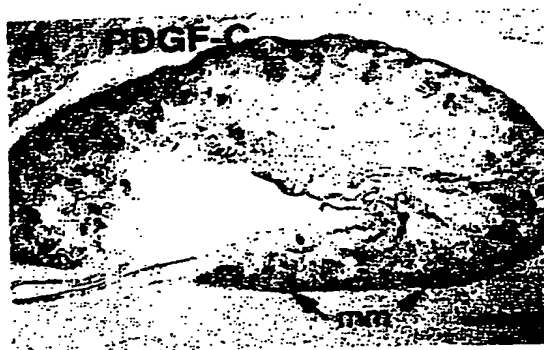
Fig. 27A                           Fig. 27B



Fig. 27C                           Fig. 27D



Fig. 27E                           Fig. 27F

Fig. 28A

Fig. 28B
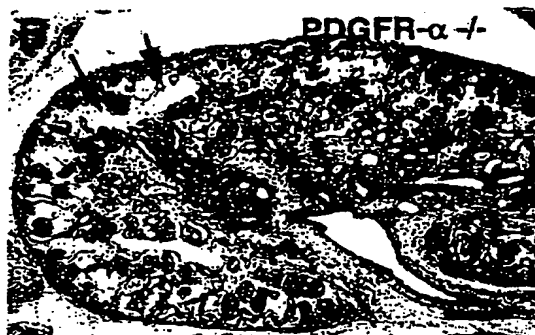


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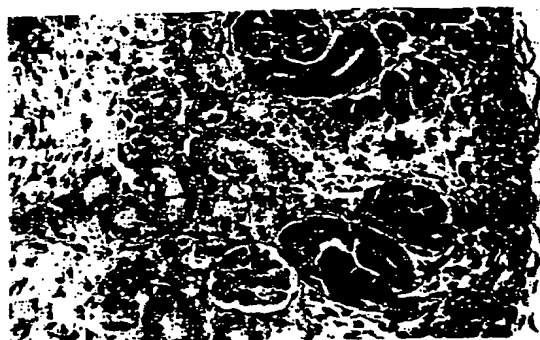
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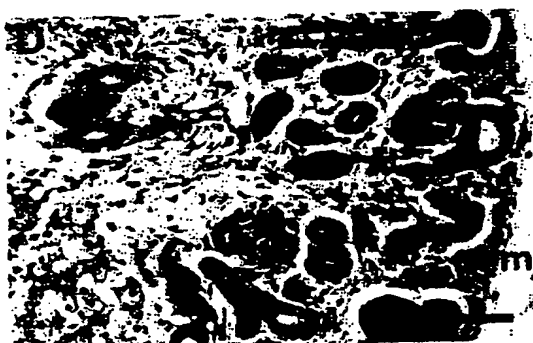


Fig. 28E

Fig. 28F
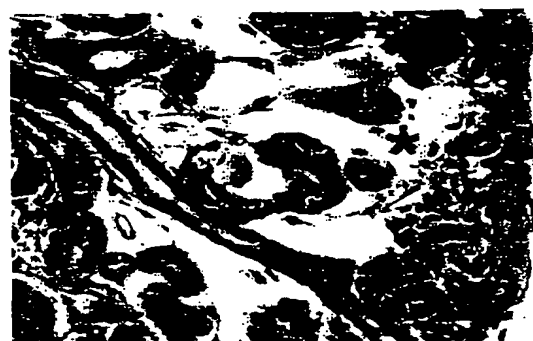
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ctggagcacc atgaggagtg tgactgtgtg tgcagaggga gcacaggagg atagccgcat 1080
caccaccagc agctcttgcc cagagctgtg cagtgcagtg ctgattcta ttagagaacg 1140
tatgcgttat ctccatcctt aatctcagtt gtttgcttca aggacctttc atcttcagga 1200
tttacagtgc attctgaaag aggagacatc aaacagaatt aggagttgtg caacagctct 1260
tttgagagga ggcctaaagg acaggagaaa aggtcttcaa tcgtggaaag aaaattaaat 1320
gttgtattaa atagatcacc agctagtttc agagttacca tgtacgtatt ccactagctg 1380
ggttctgtat ttcagttctt tcgatacggc ttagggtaat gtcagtacag gaaaaaaact 1440
gtgcaagtga gcacctgatt ccgttgcctt gcttaactct aaagctccat gtcctgggcc 1500
taaaatcgta taaaatctgg attttttttt tttttttgc tcatattcac atatgtaaac 1560
cagaacattc tatgtactac aaacctggtt tttaaaaagg aactatgttg ctatgaatta 1620
aacttgtgtc rtgctgatag gacagactgg atttttcata tttcttatta aaatttctgc 1680
catttagaag aagagaacta cattcatggt ttggaagaga taaacctgaa aagaagagtg 1740
gccttatctt cactttatcg ataagtcagt ttatttgttt cattgtgtac attttttatat 1800
tctccttttg acattataac tgttggcttt tctaatcttg ttaaatatat ctattttac 1860
caaaggtatt taatattctt ttttatgaca acttagatca actattttta gcttggtaaa 1920
tttttctaaa cacaattgtt atagccagag aacaaagat ggatataaaa atattgttgc 1980
cctggacaaa aatacatgta tntccatccc ggaatggtgc tagagttgga ttaaacctgc 2040
attttaaaaa acctgaattg ggaanggaan ttggtaaggt tggccaaanc tttttgaaa 2100
ataattaa                                                        2108
```

<210> 3
<211> 345
<212> PRT
<213> Homo sapiens

&lt;400&gt; 3

```
Met Ser Leu Phe Gly Leu Leu Leu Val Thr Ser Ala Leu Ala Gly Gln
 1               5                  10                  15

Arg Arg Gly Thr Gln Ala Glu Ser Asn Leu Ser Ser Lys Phe Gln Phe
            20                  25                  30

Ser Ser Asn Lys Glu Gln Asn Gly Val Gln Asp Pro Gln His Glu Arg
            35                  40                  45

Ile Ile Thr Val Ser Thr Asn Gly Ser Ile His Ser Pro Arg Phe Pro
        50                  55                  60

His Thr Tyr Pro Arg Asn Thr Val Leu Val Trp Arg Leu Val Ala Val
 65                  70                  75                  80

Glu Glu Asn Val Trp Ile Gln Leu Thr Phe Asp Glu Arg Phe Gly Leu
                85                  90                  95

Glu Asp Pro Glu Asp Asp Ile Cys Lys Tyr Asp Phe Val Glu Val Glu
            100                 105                 110

Glu Pro Ser Asp Gly Thr Ile Leu Gly Arg Trp Cys Gly Ser Gly Thr
            115                 120                 125

Val Pro Gly Lys Gln Ile Ser Lys Gly Asn Gln Ile Arg Ile Arg Phe
    130                 135                 140

Val Ser Asp Glu Tyr Phe Pro Ser Glu Pro Gly Phe Cys Ile His Tyr
145                 150                 155                 160

Asn Ile Val Met Pro Gln Phe Thr Glu Ala Val Ser Pro Ser Val Leu
                165                 170                 175

Pro Pro Ser Ala Leu Pro Leu Asp Leu Leu Asn Asn Ala Ile Thr Ala
            180                 185                 190

Phe Ser Thr Leu Glu Asp Leu Ile Arg Tyr Leu Glu Pro Glu Arg Trp
        195                 200                 205

Gln Leu Asp Leu Glu Asp Leu Tyr Arg Pro Thr Trp Gln Leu Leu Gly
    210                 215                 220

Lys Ala Phe Val Phe Gly Arg Lys Ser Arg Val Val Asp Leu Asn Leu
225                 230                 235                 240

Leu Thr Glu Glu Val Arg Leu Tyr Ser Cys Thr Pro Arg Asn Phe Ser
```

3

```
                    245                    250                    255

Val Ser Ile Arg Glu Glu Leu Lys Arg Thr Asp Thr Ile Phe Trp Pro
             260                    265                    270

Gly Cys Leu Leu Val Lys Arg Cys Gly Gly Asn Cys Ala Cys Cys Leu
         275                    280                    285

His Asn Cys Asn Glu Cys Gln Cys Val Pro Ser Lys Val Thr Lys Lys
     290                    295                    300

Tyr His Glu Val Leu Gln Leu Arg Pro Lys Thr Gly Val Arg Gly Leu
305                    310                    315                    320

His Lys Ser Leu Thr Asp Val Ala Leu Glu His His Glu Glu Cys Asp
             325                    330                    335

Cys Val Cys Arg Gly Ser Thr Gly Gly
             340                    345
```

```
<210> 4
<211> 1536
<212> DNA
<213> Homo sapiens

<400> 4
cgggtaaatt ccagttttcc agcaacaagg aacagaacgg agtacaagat cctcagcatg 60
agagaattat tactgtgtct actaatggaa gtattcacag cccaaggttt cctcatactt 120
atccaagaaa tacggtcttg gtatggagat tagtagcagt agaggaaaat gtatggatac 180
aacttacgtt tgatgaaaga tttgggcttg aagacccaga agatgacata tgcaagtatg 240
attttgtaga agttgaggaa cccagtgatg gaactatatt agggcgctgg tgtggttctg 300
gtactgtacc aggaaaacag atttctaaag gaaatcaaat taggataaga tttgtatctg 360
atgaatattt ccttctgaa ccagggttct gcatccacta caacattgtc atgccacaat 420
tcacagaagc tgtgagtcct tcagtgctac ccccttcagc tttgccactg acctgctta 480
ataatgctat aactgccttt agtaccttgg aagaccttat tcgatatctt gaaccagaga 540
gatggcagtt ggacttagaa gatctatata ggccaacttg caacttctt ggcaaggctt 600
ttgttttttgg aagaaaatcc agagtggtgg atctgaacct tctaacagag gaggtaagat 660
tatacagctg cacacctcgt aacttctcag tgtccataag ggaagaacta aagagaaccg 720
ataccatttt ctggccaggt tgtctcctgg ttaaacgctg tggtgggaac tgtgcctgtt 780
gtctccacaa ttgcaatgaa tgtcaatgtg tcccaagcaa agttactaaa aaataccacg 840
aggtccttca gttgagacca aasaccggtg tcaggggatt gcacaaatca ctcaccgacg 900
tggccctgga gcaccatgag gagtgtgact gtgtgtgcag agggagcaca ggaggatagc 960
cgcatcacca ccagcagctc ttgcccagag ctgtgcagtg cagtggctga ttctattaga 1020
gaacgtatgc gttatctcca tccttaatct cagttgtttg cttcaaggac ctttcatctt 1080
caggatttac agtgcattct gaaagaggag acatcaaaca gaattaggag ttgtgcaaca 1140
gctctttga gaggaggcct aaaggacagg agaaaaggtc ttcaatcgtg gaaagaaaat 1200
taaatgttgt attaaataga tcaccagcta gtttcagagt taccatgtac gtattccact 1260
```

4

```
agctgggttc tgtatttcag ttctttcgat acggcttagg gtaatgtcag tacaggaaaa 1320
aaactgtgca agtgagcacc tgattccgtt gccttgctta actctaaagc tccatgtcct 1380
gggcctaaaa tcgtataaaa tctggatttt tttttttttt tttgctcata ttcacatatg 1440
taaaccagaa cattctatgt actacaaacc tggttttta a aaaggaacta tgttgctatg 1500
aattaaactt gtgtcatgct gataggacag actgga                           1536
```

<210> 5
<211> 318
<212> PRT
<213> Homo sapiens

<400> 5

```
Gly Lys Phe Gln Phe Ser Ser Asn Lys Glu Gln Asn Gly Val Gln Asp
 1               5                  10                  15

Pro Gln His Glu Arg Ile Ile Thr Val Ser Thr Asn Gly Ser Ile His
            20                  25                  30

Ser Pro Arg Phe Pro His Thr Tyr Pro Arg Asn Thr Val Leu Val Trp
        35                  40                  45

Arg Leu Val Ala Val Glu Glu Asn Val Trp Ile Gln Leu Thr Phe Asp
    50                  55                  60

Glu Arg Phe Gly Leu Glu Asp Pro Glu Asp Asp Ile Cys Lys Tyr Asp
 65                  70                  75                  80

Phe Val Glu Val Glu Glu Pro Ser Asp Gly Thr Ile Leu Gly Arg Trp
                85                  90                  95

Cys Gly Ser Gly Thr Val Pro Gly Lys Gln Ile Ser Lys Gly Asn Gln
            100                 105                 110

Ile Arg Ile Arg Phe Val Ser Asp Glu Tyr Phe Pro Ser Glu Pro Gly
            115                 120                 125

Phe Cys Ile His Tyr Asn Ile Val Met Pro Gln Phe Thr Glu Ala Val
    130                 135                 140

Ser Pro Ser Val Leu Pro Pro Ser Ala Leu Pro Leu Asp Leu Leu Asn
145                 150                 155                 160

Asn Ala Ile Thr Ala Phe Ser Thr Leu Glu Asp Leu Ile Arg Tyr Leu
                165                 170                 175

Glu Pro Glu Arg Trp Gln Leu Asp Leu Glu Asp Leu Tyr Arg Pro Thr
            180                 185                 190
```

```
Trp Gln Leu Leu Gly Lys Ala Phe Val Phe Gly Arg Lys Ser Arg Val
        195             200             205

Val Asp Leu Asn Leu Leu Thr Glu Glu Val Arg Leu Tyr Ser Cys Thr
    210             215             220

Pro Arg Asn Phe Ser Val Ser Ile Arg Glu Glu Leu Lys Arg Thr Asp
225             230             235             240

Thr Ile Phe Trp Pro Gly Cys Leu Leu Val Lys Arg Cys Gly Gly Asn
            245             250             255

Cys Ala Cys Cys Leu His Asn Cys Asn Glu Cys Gln Cys Val Pro Ser
        260             265             270

Lys Val Thr Lys Lys Tyr His Glu Val Leu Gln Leu Arg Pro Lys Thr
    275             280             285

Gly Val Arg Gly Leu His Lys Ser Leu Thr Asp Val Ala Leu Glu His
    290             295             300

His Glu Glu Cys Asp Cys Val Cys Arg Gly Ser Thr Gly Gly
305             310             315
```

```
<210> 6
<211> 1474
<212> DNA
<213> Murinae gen. sp.

<400> 6
cacctggaga cacagaagag ggctctagga aaaattttgg atggggatta tgtggaaact 60
accctgcgat tctctgctgc cagagccggc caggcgcttc caccgcagcg cagcctttcc 120
ccgggctggg ctgagccttg gagtcgtcgc ttccccagtg cccgccgcga gtgagccctc 180
gccccagtca gccaaatgct cctcctcggc ctcctcctgc tgacatctgc cctggccggc 240
caaagaacgg ggactcgggc tgagtccaac ctgagcagca agttgcagct ctccagcgac 300
aaggaacaga acggagtgca agatccccgg catgagagag ttgtcactat atctggtaat 360
gggagcatcc acagcccgaa gtttcctcat acgtacccaa gaaatatggt gctggtgtgg 420
agattagttg cagtagatga aaatgtgcgg atccagctga catttgatga gagatttggg 480
ctggaagatc cagaagacga tatatgcaag tatgattttg tagaagttga ggagcccagt 540
gatggaagtg tttaggacg ctggtgtggt ctgggactg tgccaggaaa gcagacttct 600
aaaggaaatc atatcaggat aagatttgta tctgatgagt attttccatc tgaacccgga 660
ttctgcatcc actacagtat tatcatgcca caagtcacag aaaccacgag tccttcggtg 720
ttgcccccttt catctttgtc attggacctg ctcaacaatg ctgtgactgc cttcagtacc 780
ttggaagagc tgattcggta cctagagcca gatcgatggc aggtggactt ggacagcctc 840
tacaagccaa catggcagct tttgggcaag gcttcctgt atgggaaaaa aagcaaagtg 900
gtgaatctga atctcctcaa ggaagaggta aaactctaca gctgcacacc ccggaacttc 960
tcagtgtcca tacgggaaga gctaaagagg acagatacca tattctggcc aggttgtctc 1020
```

```
ctggtcaagc gctgtggagg aaattgtgcc tgttgtctcc ataattgcaa tgaatgtcag 1080
tgtgtcccac gtaaagttac aaaaaagtac catgaggtcc ttcagttgag accaaaaact 1140
ggagtcaagg gattgcataa gtcactcact gatgtggctc tggaacacca cgaggaatgt 1200
gactgtgtgt gtagaggaaa cgcaggaggg taactgcagc cttcgtagca gcacacgtga 1260
gcactggcat tctgtgtacc cccacaagca accttcatcc ccaccagcgt tggccgcagg 1320
gctctcagct gctgatgctg gctatggtaa agatcttact cgtctccaac caaattctca 1380
gttgtttgct tcaatagcct tcccctgcag gacttcaagt gtcttctaaa agaccagagg 1440
caccaanagg agtcaatcac aaagcactgc accg                             1474
```

<210> 7
<211> 345
<212> PRT .
<213> Murinae gen. sp.

<400> 7

```
Met Leu Leu Leu Gly Leu Leu Leu Leu Thr Ser Ala Leu Ala Gly Gln
  1               5                  10                  15

Arg Thr Gly Thr Arg Ala Glu Ser Asn Leu Ser Ser Lys Leu Gln Leu
             20                  25                  30

Ser Ser Asp Lys Glu Gln Asn Gly Val Gln Asp Pro Arg His Glu Arg
         35                  40                  45

Val Val Thr Ile Ser Gly Asn Gly Ser Ile His Ser Pro Lys Phe Pro
     50                  55                  60

His Thr Tyr Pro Arg Asn Met Val Leu Val Trp Arg Leu Val Ala Val
· 65                  70                  75                  80

Asp Glu Asn Val Arg Ile Gln Leu Thr Phe Asp Glu Arg Phe Gly Leu
                 85                  90                  95

Glu Asp Pro Glu Asp Asp Ile Cys Lys Tyr Asp Phe Val Glu Val Glu
             100                 105                 110

Glu Pro Ser Asp Gly Ser Val Leu Gly Arg Trp Cys Gly Ser Gly Thr
         115                 120                 125

Val Pro Gly Lys Gln Thr Ser Lys Gly Asn His Ile Arg Ile Arg Phe
     130                 135                 140

Val Ser Asp Glu Tyr Phe Pro Ser Glu Pro Gly Phe Cys Ile His Tyr
145                 150                 155                 160

Ser Ile Ile Met Pro Gln Val Thr Glu Thr Thr Ser Pro Ser Val Leu
                 165                 170                 175
```

Pro Pro Ser Ser Leu Ser Leu Asp Leu Leu Asn Asn Ala Val Thr Ala
              180                     185                 190

Phe Ser Thr Leu Glu Glu Leu Ile Arg Tyr Leu Glu Pro Asp Arg Trp
         195                 200                 205

Gln Val Asp Leu Asp Ser Leu Tyr Lys Pro Thr Trp Gln Leu Leu Gly
    210                 215                 220

Lys Ala Phe Leu Tyr Gly Lys Lys Ser Lys Val Val Asn Leu Asn Leu
225                 230                 235                 240

Leu Lys Glu Glu Val Lys Leu Tyr Ser Cys Thr Pro Arg Asn Phe Ser
              245                 250                 255

Val Ser Ile Arg Glu Glu Leu Lys Arg Thr Asp Thr Ile Phe Trp Pro
              260                 265                 270

Gly Cys Leu Leu Val Lys Arg Cys Gly Gly Asn Cys Ala Cys Cys Leu
         275                 280                 285

His Asn Cys Asn Glu Cys Gln Cys Val Pro Arg Lys Val Thr Lys Lys
    290                 295                 300

Tyr His Glu Val Leu Gln Leu Arg Pro Lys Thr Gly Val Lys Gly Leu
305                 310                 315                 320

His Lys Ser Leu Thr Asp Val Ala Leu Glu His His Glu Glu Cys Asp
              325                 330                 335

Cys Val Cys Arg Gly Asn Ala Gly Gly
         340                 345


<210> 8
<211> 192
<212> PRT
<213> Homo sapiens

<400> 8
Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
  1               5                 10                  15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
              20                 25                  30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
         35                 40                  45

8

```
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
    50              55              60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65              70              75              80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
            85              90              95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
            100             105             110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
        115             120             125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly
    130             135             140

Pro Cys Ser Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln
145             150             155             160

Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg
            165             170             175

Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
            180             185             190
```

```
<210> 9
<211> 170
<212> PRT
<213> Homo sapiens

<400> 9
Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly
  1           5              10              15

Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly
            20              25              30

Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly
        35              40              45

Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu
```

                    50                         55                         60

Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu
65                         70                    75                         80

Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu Asp Leu His Cys Val Pro
                    85                    90                         95

Val Glu Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
                    100                   105                        110

Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys
                    115                   120                        125

Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu Arg Arg Arg Pro
               130                   135                    140

Lys Gly Arg Gly Lys Arg Arg Arg Glu Asn Gln Arg Pro Thr Asp Cys
145                   150                        155                        160

His Leu Cys Gly Asp Ala Val Pro Arg Arg
               165                   170


<210> 10
<211> 188
<212> PRT
<213> Homo sapiens

<400> 10
Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
1                   5                    10                        15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
               20                   25                        30

Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
               35                   40                        45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
          50                   55                    60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
65                   70                    75                         80

Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
               85                   90                        95

Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
          100              105              110

Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
          115              120              125

Lys Asp Ser Ala Val Lys Pro Asp Ser Pro Arg Pro Leu Cys Pro Arg
     130              135              140

Cys Thr Gln His His Gln Arg Pro Asp Pro Arg Thr Cys Arg Cys Arg
145              150              155              160

Cys Arg Arg Arg Ser Phe Leu Arg Cys Gln Gly Arg Gly Leu Glu Leu
               165              170              175

Asn Pro Asp Thr Cys Arg Cys Arg Lys Leu Arg Arg
          180              185


<210> 11
<211> 133
<212> PRT
<213> Homo sapiens

<400> 11
Met Lys Leu Leu Val Gly Ile Leu Val Ala Val Cys Leu His Gln Tyr
  1               5               10              15

Leu Leu Asn Ala Asp Ser Asn Thr Lys Gly Trp Ser Glu Val Leu Lys
          20              25              30

Gly Ser Glu Cys Lys Pro Arg Pro Ile Val Val Pro Val Ser Glu Thr
          35              40              45

His Pro Glu Leu Thr Ser Gln Arg Phe Asn Pro Pro Cys Val Thr Leu
     50              55              60

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Ser Leu Glu Cys Val Pro
65              70              75              80

Thr Glu Glu Val Asn Val Ser Met Glu Leu Leu Gly Ala Ser Gly Ser
               85              90              95

Gly Ser Asn Gly Met Gln Arg Leu Ser Phe Val Glu His Lys Lys Cys
          100              105              110

Asp Cys Arg Pro Arg Phe Thr Thr Thr Pro Pro Thr Thr Thr Arg Pro
          115              120              125

11

Pro Arg Arg Arg Arg
    130


<210> 12
<211> 419
<212> PRT
<213> Homo sapiens

<400> 12
Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala
  1                 5                  10                  15


Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe
                20                  25                  30


Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala
            35                  40                  45


Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser
        50                  55                  60


Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met
65                  70                  75                  80


Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln
                85                  90                  95


Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala
            100                 105                 110


His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys
            115                 120                 125


Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe
        130                 135                 140


Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr
145                 150                 155                 160


Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr
                165                 170                 175


Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu
            180                 185                 190


Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser

```
              195                    200                    205

     Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile
         210                    215                    220

     Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn
     225                    230                    235                    240

     Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys
                     245                    250                    255

     Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser
                 260                    265                    270

     Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu
                 275                    280                    285

     Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys
         290                    295                    300

     Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys
     305                    310                    315                    320

     Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu
                     325                    330                    335

     Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro
                 340                    345                    350

     Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys
                 355                    360                    365

     Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr
         370                    375                    380

     Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser
     385                    390                    395                    400

     Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro
                     405                    410                    415

     Gln Met Ser
```

```
<210> 13
<211> 358
<212> PRT
```

&lt;213&gt; Homo sapiens

&lt;400&gt; 13
Met Tyr Gly Glu Trp Gly Met Gly Asn Ile Leu Met Met Phe His Val
 1               5                   10                  15

Tyr Leu Val Gln Gly Phe Arg Ser Glu His Gly Pro Val Lys Asp Phe
                20                  25                  30

Ser Phe Glu Arg Ser Ser Arg Ser Met Leu Glu Arg Ser Glu Gln Gln
            35                  40                  45

Ile Arg Ala Ala Ser Ser Leu Glu Glu Leu Leu Gln Ile Ala His Ser
        50                  55                  60

Glu Asp Trp Lys Leu Trp Arg Cys Arg Leu Lys Leu Lys Ser Leu Ala
65                  70                  75                      80

Ser Met Asp Ser Arg Ser Ala Ser His Arg Ser Thr Arg Phe Ala Ala
                85                  90                  95

Thr Phe Tyr Asp Thr Glu Thr Leu Lys Val Ile Asp Glu Glu Trp Gln
            100                 105                 110

Arg Thr Gln Cys Ser Pro Arg Glu Thr Cys Val Glu Val Ala Ser Glu
            115                 120                 125

Leu Gly Lys Thr Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Asn Val
        130                 135                 140

Phe Arg Cys Gly Gly Cys Cys Asn Glu Glu Gly Val Met Cys Met Asn
145                 150                 155                 160

Thr Ser Thr Ser Tyr Ile Ser Lys Gln Leu Phe Glu Ile Ser Val Pro
                165                 170                 175

Leu Thr Ser Val Pro Glu Leu Val Pro Val Lys Ile Ala Asn His Thr
            180                 185                 190

Gly Cys Lys Cys Leu Pro Thr Gly Pro Arg His Pro Tyr Ser Ile Ile
            195                 200                 205

Arg Arg Ser Ile Gln Thr Pro Glu Glu Asp Glu Cys Pro His Ser Lys
    210                 215                 220

Lys Leu Cys Pro Ile Asp Met Leu Trp Asp Asn Thr Lys Cys Lys Cys
225                 230                 235                 240

14

```
Val Leu Gln Asp Glu Thr Pro Leu Pro Gly Thr Glu Asp His Ser Tyr
              245             250             255

Leu Gln Glu Pro Thr Leu Cys Gly Pro His Met Thr Phe Asp Glu Asp
              260             265             270

Arg Cys Glu Cys Val Cys Lys Ala Pro Cys Pro Gly Asp Leu Ile Gln
          275             280             285

His Pro Glu Asn Cys Ser Cys Phe Glu Cys Lys Glu Ser Leu Glu Ser
      290             295             300

Cys Cys Gln Lys His Lys Ile Phe His Pro Asp Thr Cys Ser Cys Glu
305             310             315             320

Asp Arg Cys Pro Phe His Thr Arg Thr Cys Ala Ser Arg Lys Pro Ala
              325             330             335

Cys Gly Lys His Trp Arg Phe Pro Lys Glu Thr Arg Ala Gln Gly Leu
              340             345             350

Tyr Ser Gln Glu Asn Pro
          355
```

```
<210> 14
<211> 211
<212> PRT
<213> Homo sapiens

<400> 14
Met Arg Thr Leu Ala Cys Leu Leu Leu Gly Cys Gly Tyr Leu Ala
  1           5             10                  15

His Val Leu Ala Glu Glu Ala Glu Ile Pro Arg Glu Val Ile Glu Arg
              20              25                  30

Leu Ala Arg Ser Gln Ile His Ser Ile Arg Asp Leu Gln Arg Leu Leu
          35              40              45

Glu Ile Asp Ser Val Gly Ser Glu Asp Ser Leu Asp Thr Ser Leu Arg
      50              55              60

Ala His Gly Val His Ala Thr Lys His Val Pro Glu Lys Arg Pro Leu
 65                 70              75                  80

Pro Ile Arg Arg Lys Arg Ser Ile Glu Glu Ala Val Pro Ala Val Cys
              85              90                  95
```

                                    15

Lys Thr Arg Thr Val Ile Tyr Glu Ile Pro Arg Ser Gln Val Asp Pro
            100             105             110

Thr Ser Ala Asn Phe Leu Ile Trp Pro Pro Cys Val Glu Val Lys Arg
            115             120             125

Cys Thr Gly Cys Cys Asn Thr Ser Ser Val Lys Cys Gln Pro Ser Arg
        130             135             140

Val His His Arg Ser Val Lys Val Ala Lys Val Glu Tyr Val Arg Lys
145             150             155             160

Lys Pro Lys Leu Lys Glu Val Gln Val Arg Leu Glu Glu His Leu Glu
                165             170             175

Cys Ala Cys Ala Thr Thr Ser Leu Asn Pro Asp Tyr Arg Glu Glu Asp
            180             185             190

Thr Gly Arg Pro Arg Glu Ser Gly Lys Lys Arg Lys Arg Lys Arg Leu
        195             200             205

Lys Pro Thr
    210


<210> 15
<211> 241
<212> PRT
<213> Homo sapiens

<400> 15
Met Asn Arg Cys Trp Ala Leu Phe Leu Ser Leu Cys Cys Tyr Leu Arg
  1             5               10              15

Leu Val Ser Ala Glu Gly Asp Pro Ile Pro Glu Glu Leu Tyr Glu Met
            20              25              30

Leu Ser Asp His Ser Ile Arg Ser Phe Asp Asp Leu Gln Arg Leu Leu
            35              40              45

His Gly Asp Pro Gly Glu Glu Asp Gly Ala Glu Leu Asp Leu Asn Met
        50              55              60

Thr Arg Ser His Ser Gly Gly Glu Leu Glu Ser Leu Ala Arg Gly Arg
65              70              75              80

Arg Ser Leu Gly Ser Leu Thr Ile Ala Glu Pro Ala Met Ile Ala Glu

16

```
                    85                      90                      95

Cys Lys Thr Arg Thr Glu Val Phe Glu Ile Ser Arg Arg Leu Ile Asp
            100                     105                     110

Arg Thr Asn Ala Asn Phe Leu Val Trp Pro Pro Cys Val Glu Val Gln
            115                     120                     125

Arg Cys Ser Gly Cys Cys Asn Asn Arg Asn Val Gln Cys Arg Pro Thr
            130                     135                     140

Gln Val Gln Leu Arg Pro Val Gln Val Arg Lys Ile Glu Ile Val Arg
145                     150                     155                     160

Lys Lys Pro Ile Phe Lys Lys Ala Thr Val Thr Leu Glu Asp His Leu
                165                     170                     175

Ala Cys Lys Cys Glu Thr Val Ala Ala Ala Arg Pro Val Thr Arg Ser
            180                     185                     190

Pro Gly Gly Ser Gln Glu Gln Arg Ala Lys Thr Pro Gln Thr Arg Val
            195                     200                     205

Thr Ile Arg Thr Val Arg Val Arg Arg Pro Pro Lys Gly Lys His Arg
            210                     215                     220

Lys Phe Lys His Thr His Asp Lys Thr Ala Leu Lys Glu Thr Leu Gly
225                     230                     235                     240

Ala
```

```
<210> 16
<211> 182
<212> PRT
<213> Homo sapiens

<400> 16
Met Pro Gln Phe Thr Asp Cys Val Cys Arg Gly Ser Thr Gly Gly Glu
  1               5                   10                  15

Ala Val Ser Pro Ser Val Leu Pro Pro Ser Ala Leu Pro Leu Asp Leu
            20                  25                  30

Leu Asn Asn Ala Ile Thr Ala Phe Ser Thr Leu Glu Asp Leu Ile Arg
            35                  40                  45
```

Tyr Leu Glu Pro Glu Arg Trp Gln Leu Asp Leu Glu Asp Leu Tyr Arg
      50                      55                  60

Pro Thr Trp Gln Leu Leu Gly Lys Ala Phe Val Phe Gly Arg Lys Ser
 65                      70                  75                  80

Arg Val Val Asp Leu Asn Leu Leu Thr Glu Glu Val Arg Leu Tyr Ser
                  85                  90                  95

Cys Thr Pro Arg Asn Phe Ser Val Ser Ile Arg Glu Glu Leu Lys Arg
              100                 105                 110

Thr Asp Thr Ile Phe Trp Pro Gly Cys Leu Leu Val Lys Arg Cys Gly
          115                 120                 125

Gly Asn Cys Ala Cys Cys Leu His Asn Cys Asn Glu Cys Gln Cys Val
      130                 135                 140

Pro Ser Lys Val Thr Lys Lys Tyr His Glu Val Leu Gln Leu Arg Pro
145                 150                 155                 160

Lys Thr Gly Val Arg Gly Leu His Lys Ser Leu Thr Asp Val Ala Leu
              165                 170                 175

Glu His His Glu Glu Cys
              180


<210> 17
<211> 182
<212> PRT
<213> Murinae gen. sp.

<400> 17
Met Pro Gln Val Thr Glu Thr Thr Ser Pro Ser Val Leu Pro Pro Ser
 1               5                   10                  15

Ser Leu Ser Leu Asp Leu Leu Asn Asn Ala Val Thr Ala Phe Ser Thr
              20                  25                  30

Leu Glu Glu Leu Ile Arg Tyr Leu Glu Pro Asp Arg Trp Gln Val Asp
          35                  40                  45

Leu Asp Ser Leu Tyr Lys Pro Thr Trp Gln Leu Asp Cys Val Cys Arg
      50                  55                  60

Gly Asn Ala Gly Gly Leu Gly Lys Ala Phe Leu Tyr Gly Lys Lys Ser
 65                  70                  75                  80

18

```
Lys Val Val Asn Leu Asn Leu Leu Lys Glu Glu Val Lys Leu Tyr Ser
              85                   90              95

Cys Thr Pro Arg Asn Phe Ser Val Ser Ile Arg Glu Glu Leu Lys Arg
          100                 105             110

Thr Asp Thr Ile Phe Trp Pro Gly Cys Leu Leu Val Lys Arg Cys Gly
          115                 120             125

Gly Asn Cys Ala Cys Cys Leu His Asn Cys Asn Glu Cys Gln Cys Val
    130                 135             140

Pro Arg Lys Val Thr Lys Lys Tyr His Glu Val Leu Gln Leu Arg Pro
145                 150             155                 160

Lys Thr Gly Val Lys Gly Leu His Lys Ser Leu Thr Asp Val Ala Leu
              165                 170                 175

Glu His His Glu Glu Cys
          180
```

```
<210> 18
<211> 117
<212> PRT
<213> Murinae gen. sp.

<400> 18
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Glu Arg Val Val Thr Ile Ser Gly Asn Gly Ser Ile His Ser Pro Lys
  1               5                   10              15

Phe Pro His Thr Tyr Pro Arg Asn Met Val Leu Val Trp Arg Leu Val
              20                  25              30

Ala Val Asp Glu Asn Val Arg Ile Gln Leu Thr Phe Asp Glu Arg Phe
          35                  40                  45

Gly Leu Glu Asp Pro Glu Asp Asp Ile Cys Lys Tyr Asp Phe Val Glu
    50                  55                  60

Val Glu Glu Pro Ser Asp Gly Ser Val Leu Gly Arg Trp Cys Gly Ser
65                  70                  75                  80

Gly Thr Val Pro Gly Lys Gln Thr Ser Lys Gly Asn Met Ile Arg Ile
              85                  90                  95

Arg Phe Val Ser Asp Glu Tyr Phe Pro Ser Glu Pro Gly Phe Cys Ile
```

```
            100                     105                     110
```

His Tyr Ser Ile Ile
        115


<210> 19
<211> 117
<212> PRT
<213> Homo sapiens

<400> 19
Glu Arg Ile Ile Thr Val Ser Thr Asn Gly Ser Ile His Ser Pro Arg
 1               5                  10                  15

Phe Pro His Thr Tyr Pro Arg Asn Thr Val Leu Val Trp Arg Leu Val
            20                  25                  30

Ala Val Glu Glu Asn Val Trp Ile Gln Leu Thr Phe Asp Glu Arg Phe
        35                  40                  45

Gly Leu Glu Asp Pro Glu Asp Asp Ile Cys Lys Tyr Asp Phe Val Glu
        50                  55                  60

Val Glu Glu Pro Ser Asp Gly Thr Ile Leu Gly Arg Trp Cys Gly Ser
 65                  70                  75                  80

Gly Thr Val Pro Gly Lys Gln Ile Ser Lys Gly Asn Gln Ile Arg Ile
                85                  90                  95

Arg Phe Val Ser Asp Glu Tyr Phe Pro Ser Glu Pro Gly Phe Cys Ile
            100                 105                 110

His Tyr Asn Ile Val
        115


<210> 20
<211> 113
<212> PRT
<213> Homo sapiens

<400> 20
Cys Gly Glu Thr Leu Gln Asp Ser Thr Gly Asn Phe Ser Ser Pro Glu
 1               5                  10                  15

Tyr Pro Asn Gly Tyr Ser Ala His Met His Cys Val Trp Arg Ile Ser
            20                  25                  30

Val Thr Pro Gly Glu Lys Ile Ile Leu Asn Phe Thr Ser Leu Asp Leu
        35                  40                  45

Tyr Arg Ser Arg Leu Cys Trp Tyr Asp Tyr Val Glu Val Arg Asp Gly
    50                  55                  60

Phe Trp Arg Lys Ala Pro Leu Arg Gly Arg Phe Cys Gly Ser Lys Leu
65                  70                  75                  80

Pro Glu Pro Ile Val Ser Thr Asp Ser Arg Leu Trp Val Glu Phe Arg
                85                  90                  95

Ser Ser Ser Asn Trp Val Gly Lys Gly Phe Phe Ala Val Tyr Glu Ala
            100                 105                 110

Ile


<210> 21
<211> 112
<212> PRT
<213> Homo sapiens

<400> 21
Cys Gly Gly Asp Val Lys Lys Asp Tyr Gly His Ile Gln Ser Pro Asn
  1             5                   10                  15

Tyr Pro Asp Asp Tyr Arg Pro Ser Lys Val Cys Ile Trp Arg Ile Gln
            20                  25                  30

Val Ser Glu Gly Phe His Val Gly Leu Thr Phe Gln Ser Phe Glu Ile
        35                  40                  45

Glu Arg Met Asp Ser Cys Ala Tyr Asp Tyr Leu Glu Val Arg Asp Gly
    50                  55                  60

His Ser Glu Ser Ser Thr Leu Ile Gly Arg Tyr Cys Gly Tyr Glu Lys
65                  70                  75                  80

Pro Asp Asp Ile Lys Ser Thr Ser Ser Arg Leu Trp Leu Lys Phe Val
                85                  90                  95

Ser Asp Gly Ser Ile Asn Lys Ala Gly Phe Ala Val Asn Phe Phe Lys
            100                 105                 110

<210> 22
<211> 113
<212> PRT
<213> Homo sapiens.

<400> 22
Cys Gly Gly Phe Leu Thr Lys Leu Asn Gly Ser Ile Thr Ser Pro Gly
1               5                   10                  15

Trp Pro Lys Glu Tyr Pro Pro Asn Lys Asn Cys Ile Trp Gln Leu Val
                20                  25                  30

Ala Pro Thr Gln Tyr Arg Ile Ser Leu Gln Phe Asp Phe Phe Glu Thr
            35                  40                  45

Glu Gly Asn Asp Val Cys Lys Tyr Asp Phe Val Glu Val Arg Ser Gly
    50                  55                  60

Leu Thr Ala Asp Ser Lys Leu His Gly Lys Phe Cys Gly Ser Glu Lys
65                  70                  75                  80

Pro Glu Val Ile Thr Ser Gln Tyr Asn Asn Met Arg Val Glu Pro Lys
                85                  90                  95

Ser Asp Asn Thr Val Ser Lys Lys Gly Phe Lys Ala His Phe Phe Ser
            100                 105                 110

Glu


<210> 23
<211> 113
<212> PRT
<213> Homo sapiens

<400> 23
Gly Asp Thr Ile Lys Ile Glu Ser Pro Gly Tyr Leu Thr Ser Pro Gly
1               5                   10                  15

Tyr Pro His Ser Tyr His Pro Ser Glu Lys Cys Glu Trp Leu Ile Gln
                20                  25                  30

Ala Pro Asp Pro Tyr Gln Arg Ile Met Ile Asn Phe Asn Pro His Phe
            35                  40                  45

```
Asp Leu Glu Asp Arg Asp Cys Lys Tyr Asp Tyr Val Glu Val Phe Asp
      50              55              60

Gly Glu Asn Glu Asn Gly His Phe Arg Gly Lys Phe Cys Gly Lys Ile
 65              70              75              80

Ala Pro Pro Pro Val Val Ser Ser Gly Pro Phe Leu Phe Ile Lys Phe
              85              90              95

Val Ser Asp Tyr Glu Thr His Gly Ala Gly Phe Ser Ile Arg Tyr Glu
              100             105             110

Ile
```

```
<210> 24
<211> 119
<212> PRT
<213> Homo sapiens

<400> 24
Cys Ser Gln Asn Tyr Thr Thr Pro Ser Gly Val Ile Lys Ser Pro Gly
 1               5               10              15

Phe Pro Glu Lys Tyr Pro Asn Ser Leu Glu Cys Thr Tyr Ile Val Phe
              20              25              30

Ala Pro Lys Met Ser Glu Ile Ile Leu Glu Phe Glu Ser Phe Asp Leu
              35              40              45

Glu Pro Asp Ser Asn Pro Pro Gly Gly Met Phe Cys Arg Tyr Asp Arg
      50              55              60

Leu Glu Ile Trp Asp Gly Phe Pro Asp Val Gly Pro His Ile Gly Arg
 65              70              75              80

Tyr Cys Gly Gln Lys Thr Pro Gly Arg Ile Arg Ser Ser Ser Gly Ile
              85              90              95

Leu Ser Met Val Phe Tyr Thr Asp Ser Ala Ile Ala Lys Glu Gly Phe
              100             105             110

Ser Ala Asn Tyr Ser Val Leu
              115
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<210> 25
<211> 19
<212> DNA
<213> Homo sapiens

<400> 25
gaagttgagg aacccagtg                                                    19


<210> 26
<211> 20
<212> DNA
<213> Homo sapiens

<400> 26
cttgccaaga agttgccaag                                                   20


<210> 27
<211> 19
<212> DNA
<213> Murinae gen. sp.

<400> 27
cttcagtacc ttggaagag                                                    19


<210> 28
<211> 19
<212> DNA
<213> Murinae gen. sp.

<400> 28
cgcttgacca ggagacaac                                                    19


<210> 29
<211> 30
<212> DNA
<213> Murinae gen. sp.

<400> 29
acgtgaattc agcaagttca gcctggttaa                                        30


<210> 30
<211> 30
<212> DNA
<213> Murinae gen. sp.

<400> 30
acgtggatcc tgagtatttc ttccagggta                                        30

<210> 31
<211> 22
<212> PRT
<213> Homo sapiens

<400> 31
Cys Lys Phe Gln Phe Ser Ser Asn Lys Glu Gln Asn Gly Val Gln Asp
 1               5                   10                  15

Pro Gln His Glu Arg Cys
            20

<210> 32
<211> 21
<212> PRT
<213> Homo sapiens

<400> 32
Gly Arg Lys Ser Arg Val Val Asp Leu Asn Leu Leu Thr Glu Glu Val
 1               5                   10                  15

Arg Leu Tyr Ser Cys
            20

<210> 33
<211> 26
<212> DNA
<213> Homo sapiens

<400> 33
cgggatcccg aatccaacct gagtag                                        26

<210> 34
<211> 61
<212> DNA
<213> Homo sapiens

<400> 34
ggaattccta atggtgatgg tgatgatgtt tgtcatcgtc atctcctcct gtgctccctc 60
t                                                                  61

<210> 35
<211> 29
<212> DNA
<213> Homo sapiens

<400> 35
cggatcccgg aagaaaatcc agagtggtg                                        29


<210> 36
<211> 61
<212> DNA
<213> Homo sapiens


<400> 36
ggaattccta atggtgatgg tgatgatgtt tgtcatcgtc atctcctcct gtgctccctc 60
t                                                                      61


<210> 37
<211> 21
<212> PRT
<213> Homo sapiens


<400> 37
Gly Arg Lys Ser Arg Val Val Asp Leu Asn Leu Leu Thr Glu Glu Val
  1               5                  10                  15

Arg Leu Tyr Ser Cys
             20



<210> 38
<211> 26
<212> DNA
<213> Homo sapiens


<220>
<223> Forward PCR primer from the human PDGF-C 430 bp
      cDNA fragment encoding the CUB domain which
      includes a BamHI site


<400> 38
cgggatcccg aatccaacct gagtag                                           26


<210> 39
<211> 60
<212> DNA
<213> Homo sapiens


<220>
<223> Reverse  PCR primer from the human PDGF-C 430 bp
      cDNA fragment encoding the CUB domain which
      includes a EcoRI site and sequences coding for a
      C-terminal 6X His tag preceded by an enterokinase

site

<400> 39
ccggaattcc taatggtgat ggtgatgatg tttgtcatcg tcgtcgacaa tgttgtagtg 60

# PATENT COOPERATION TREATY

# PCT

### DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT
### (PCT Article 17(2)(a) and Rule 39)

| Applicant's or agent's file reference<br><br>1064/44740PC | IMPORTANT DECLARATION | Date of mailing (day/month/year) |
|---|---|---|
| International application No.<br><br>PCT/US99/22668 | International filing date (day/month/year)<br><br>30 SEPTEMBER 1999 | (Earliest) Priority Date (day/month/year)<br><br>30 SEPTEMBER 1998 |
| International Patent Classification (IPC) or both national classification and IPC | | |
| Applicant<br>LUDWIG INSTITUTE FOR CANCER RESEARCH | | |

This International Searching Authority hereby declares, according to Article 17(2)(a), that no international search report will be established on the international application for the reasons indicated below.

1. ☐ The subject matter of the international application relates to:
    - a. ☐ scientific theories.
    - b. ☐ mathematical theories.
    - c. ☐ plant varieties.
    - d. ☐ animal varieties.
    - e. ☐ essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes.
    - f. ☐ schemes, rules or methods of doing business.
    - g. ☐ schemes, rules or methods of performing purely mental acts.
    - h. ☐ schemes, rules or methods of playing games.
    - i. ☐ methods for treatment of the human body by surgery or therapy.
    - j. ☐ methods for treatment of the animal body by surgery or therapy.
    - k. ☐ diagnostic methods practiced on the human or animal body.
    - l. ☐ mere presentations of information.
    - m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art.

2. ☐ The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:

    ☐ the description      ☐ the claims      ☐ the drawings

3. ☒ The failure of the nucleotide and/or amino acid sequence listing to comply with the prescribed requirements prevents a meaningful search from being carried out:

    ☐ it does not comply with the prescribed standard
    ☒ it is not in the prescribed machine readable form

4. Further comments:
   The CRF (computer readable form) for this case is defective. Claims which recite SEQ ID No:s or depend therefrom which cannot be searched other than by a sequence search are held unsearchable

| Name and mailing address of the ISA/US<br><br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br><br>Facsimile No. (703) 305-3230 | Authorized officer<br><br>ELIANE LAZAR-WESLEY<br><br>Telephone No. (703) 308-0196 |
|---|---|